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Vegetables as a Major Vitamin A Source in Our Diet

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Cooperative Regional Research Project NE-116

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1. Introduction

When the classic book on carotenoids was published by Karrer and Jucker in 1950, there were 80 carotenoids which were known and of these perhaps only 35 had established chemical structures. In less than 40 years the number has leaped to a figure greater than 500. The previous 40 years' saw the discovery of a fat soluble growth factor A which was linked to nutritional blindness or a condition called xerophthalmia. In 1920 the yellow pigments of corn were also found to be somehow related to this growth factor A. In 1930 the precursor product relationship of p-carotene and retinol was understood. This led rapidly to the synthesis of vitamin A in 1947 and the synthesis of p-carotene in 1950.

It has only been in the last two or three decades that we have understood the extent of the deficiency of vitamin A and the resulting tragedy of blindness and death in many parts of the world. Many of the 73 countries listed by the Protein Calorie Advisory Group (1976) as having potentially serious vitamin A deficiency problems have not been well studied. What is alarming is that studies done in depth on particular areas indicate that in four countries in South East Asia alone one-half million children will develop some level of xerophthalmia and half of the affected will die directly or indirectly from the deficiency (McLaren, 1986). When one extrapolates to other countries of Asia, Africa and Latin America, the mortality and morbidity is staggering. The good news is that vitamin A capsules and vitamin A and provitamin A containing foods are abundant and inexpensive in many countries. The bad news is that the distribution of capsules is under the uncertainties of government control, and people are often not very teachable in regard to their diets.

In the U. S. the National Nutrition Monitoring System operated by the U. S. Departments of Agriculture, and Health and Human Services recently issued their joint nutrition monitoring evaluation on the nutritional status of the U. S. population. This unique report stated that vitamin A and protein, along with thiamin, riboflavin and niacin are "food components warranting continued public health monitoring consideration". Consumers are becoming aware of links between certain diseases and nutrients such as carotenoids and vitamin C, all of which are supplied by fruits and vegetables.

Table 1 lists the estimated distribution of the various sources of vitamin A and provitamin A in foods. It can be seen that some foods contain preformed vitamin A while fruits and vegetables contain provitamin A carotenoids. However, all the animal sources contain a significant amount of vitamin A which ultimately come from plant sources. While plants are estimated to contribute approximately 50% of the total vitamin A content of the American diet, it has been estimated that the figure is 65% in Taiwan and probably much higher in less developed countries (Simpson and Tsou,1987).

Table 1. Estimated Distribution of Sources of Vitamin A Activity in Various Foods

Source	From retinol precursors		
	From retinol	β-Carotene	Carotenoids other than β-carotene
Animal origin			
Meat & meat organs	90	10	
Poultry	70	30	
Fish & shellfish	90	10	
Eggs	70	30	
Milk & milk products	70	30	
Animal & fish oil	90	10	
Plant origin			
Cereals;			
Maize, yellow		40	60
Other		50	50
Legumes & seeds		50	50
Vegetables;			
Green vegetables		75	25
Deep yellow vegetables (carrots, etc)		85	15
Sweet potatoes, pale type		50	50
Fruits;			
Deep yellow fruits		85	15
Other fruits		75	25
Vegetable oils;			
Red palm oil		65	35
Other vegetable or seed oils		50	50

From Leung et al., (1968)

While vitamin A activity and vitamin A status are important, there is another anthropocentric consideration i.e. the color that carotenoids impart to our foods and environment. All green vegetables contain carotenoids as these compounds offer effective protection against photodynamic destruction of the plant. While we are not clear as to the function of the carotenoids in other vegetables, we certainly are aware of the fact that the quality of carrots, squash, corn, etc. is directly related to color.

Recently there is a body of evidence which suggests that the carotenoids can function in medical applications. The work by Mathews-Roth et al. (1970) from Harvard showed some time ago that the symptoms of erythropoietic porphyria could be eased by large doses of p-carotene when the pigment was deposited in the skin of patients who lacked pigmentation. More recently, carotenoids have been shown to be effective in the treatment of cancer particularly cancer of epithelial origin. p-Carotene was effective in preventing neoplastic transformations caused by various carcinogens. Other carotenoids such as canthaxanthin with no vitamin A activity were as effective as p-carotene. Thus the carotenoids may well be acting as antioxidants. Epidemiological evidence strongly suggests that they

may have a role in cancer prevention. In one study (Shekelle et al., 1981), the subjects that consumed diets rich in vegetables developed fewer lung cancers whether or not they smoked.

) The assumption is made that any carotenoid having one-half the structure of p-carotene should be vitamin A active. We could thus estimate the number of provitamin A carotenoids from the known carotenoids to be greater than 50. Of this number, only p-carotene, *y*-carotene, a-carotene, p-zeacarotene and p-cryptoxanthin are common in fruits and vegetables. Thus any method of analysis of the carotenoids for vitamin A activity must separate these individual provitamin A carotenoids from carotenoids with no activity.

Due to the chemical complexity of carotenoids and the difficulty involved in separation of complex carotenoid mixtures, the vitamin A value of fruits and vegetables has not only been incorrectly reported but also inaccurately interpreted for many years. In addition, effects of processing and enzymes on carotenoids often have not been considered and consequently vitamin A values for some fresh and canned vegetables may be erroneously reported. As will be discussed in this paper, the correct analysis of provitamin A is not always accomplished by the official methods. Even today, the vitamin A value of many fresh and processed fruits and vegetables is not accurately tabulated, including the recently revised USDA Handbooks 8-9 and 8-11 (Bureau and Bushway, 1986). Therefore, it is essential to develop state of the art analytical techniques for \provitamin A carotenoids and their isomers in both fresh 'and processed vegetables.

Additional possible errors in expressing and understanding vitamin A value of fresh and processed vegetables are due to the following several reasons. First, carotenoids are relatively stable as compared to other vitamins, but carotenoids possess a series of conjugated double bonds so that they are highly susceptible to oxidation, which is accelerated by oxygen, uv/vis light, heavy metals and high temperature. Therefore, we expect some loss of vitamin A value during processing. Nevertheless, conflicting results are often reported that some heat processed vegetables have a higher vitamin A value than the corresponding fresh (raw) commodity. This is mainly due to inaccurate analytical procedures that include biologically non-active carotenoids. Second, a large difference in carotene content among cultivars within the same species of vegetables is often reported; sometimes these differences are over ten-fold. We have to realize the fact that there is a wide variation in the amount of carotenoids within a cultivar because of maturity, location of crop growth, and the season. Therefore, if we compare one cultivar of a fresh crop with an unknown cultivar of a processed product, we cannot make an accurate statement as to the effect of processing on the provitamin A content. The third point is related to the validity of the calculation of vitamin A value of processed) vegetables in terms of their dry weight. Since the moisture content of fresh and processed products is different, calculations have often been based on dry weight in order to compare the treatments. This may be misleading because the loss of soluble solids during processing is not taken into account in the calculation. Eventually, this gives high carotenoid values in processed products. The last source of error is the effects of enzymes on carotenoids in the fresh vegetables. According to Booth (1960) and others (Edwards and Lee, 1986), some of the enzymatic systems within many chlorophyll containing plants are known to be carotene oxidizers. During analysis of fresh vegetables, they bleach carotenoids and consequently lower the vitamin A value in fresh produce (Lee and Smith, 1988).

In order to understand these facts and to respond to the needs described above, cooperative efforts are being undertaken by the regional project NE-116 for several years. Development of analytical methodology and application and effects of processing and enzymes on provitamin A carotenoids in vegetables have been carried out under this project. This paper summarizes some recent understanding on provitamin A carotenoids, bioavailability, metabolism, analytical procedures, and effects of post-harvest storage and processing on provitamin A carotenoids in vegetables.

2. Provitamin A Carotenoids

As was stated above, over 500 carotenoid structures have been reported. The various carotenoids are found in very diverse tissues such as bacteria, yeast, molds, protista and various plants and animals. Plants are able to biosynthesize the basic carotenoid molecule (usually a 40 carbon compound). Animals on the other hand can ingest the carotenoids and split provitamin A precursors to vitamin A, deposit the pigments unchanged as in the salmon or make reductive or oxidative changes. p-Carotene in theory should yield two molecules of retinol, and other carotenoids with half of the molecules resembling p-carotene should yield one molecule of retinol. As will be discussed later, this is complicated since the splitting of the molecule may not always be at the center, i.e. the 15,15' position. On the basis of structure a number of carotenoids could be sources of retinol. The number of such compounds has been estimated to be greater than 60. However, in vegetables and fruits we commonly encounter a much smaller number of compounds; p-carotene is very widely found, and to a lesser extent, other provitamin A precursors, such as a-carotene, p-zeacarotene, *y*-carotene and p-cryptoxanthin are also found.

Table 2 lists some common provitamin A and non-provitamin A carotenoids. This list combines the data presented by Bauernfeind and Zechmeister. In Table 2 it can be seen that the formation of *cis* isomers lowers the activity. The work by Sweeny and Marsh (1971) suggests that the *cis* isomer may be converted back to the all *-trans* form in the acid environment of the stomach. Recent data by Heinonen (1988) suggests that this is not the case. Both the 5,6,5',6' and the 5,6,5',8' diepoxides were made from C^{14} labeled p-carotene and were fed to vitamin A deficient rats. No C^{14}

was isolated from the liver of the rats.

It should also be mentioned that a number of studies have shown that some fish, especially when vitamin A deficient, are able to convert compounds such as astaxanthin 3,3' dihydroxy 4,4' diketo p-carotene to retino! (Al-Khalifa and Simpson, 1988).

Table 2. Representative types of carotenoids and apocarotenoids with provitamin A activity [a])

Carotenoid	Activity {%)
p-Carotene	100
Neo-p-carotene	38[b])
ct-Carotene	50-54
Neo-a-carotena	13[b])
3,4,-De hydro-p-carotene	75
3,4,3,4-Bisdehydro-p-carctene	38
y-Carotsne	42-50
7,8-Dihydro-a-carotene	20-40
p-Carotene-5',6-epoxide	21
a-Carotene-5',6-epoxide	25
p-Carotene-5,6,5',6-diepoxide	Active
3-Keto-B-carotene	52
3- H yd roxy- p-carotene	50-60
4-Hydroxy-p-carotene	48
p-Apo-2-carotenal	Active
P-Apo-8'-carotenal	72
P-Apo-10-carotenal	Active
p-Apo-12-carotenal	120
Lycopene	Inactive
Lutein	Inactive

[a]) From Bauernfeind (1972):[b]) From Zechmeister (1949).

3. Bioavailability and Metabolism of Carotenoids

The possibility that carotenoids can be functioning as compounds with their own biological activity as well as precursors for vitamin A, has resulted in metabolic research in two areas. While most of the early carotenoid work focused upon intestinal conversion of carotenoids to retinol, contemporary research has reflected more of an interest in extra-intestinal carotenoid metabolism. Several excellent reviews have already been published concerning the factors influencing the intestinal metabolism of carotenoids (Singh and Cama, 1975; Simpson and Chichester, 1981; Goodman, 1984; and Ganguly and Sastry, 1985). Emphasis in this review has therefore been placed upon discussing the most recent research concerning both carotenoid bioavailability as well as the relationship of carotenoid intake to tissue carotene uptake.

When carotenoids are consumed, they are acted upon in the intestinal mucosa. Most of the work that has been accomplished has employed p-carotene as the substrate and has demonstrated that retinal is formed as a result of cleavage of the molecule. There is still dispute as to the nature of this cleavage process. Where some argue that cleavage can be asymmetric and the initial cleavage may occur excentrally at a variety of double bonds (Ganguly and Sastry, 1985), other contend that the evidence supports primarily central cleavage by a putative 15-15' dioxygenase (Anon., 1988). The most serious obstacle in resolving this dispute is that modern separation and purification techniques have not been used to purify the enzyme or enzymes responsible for the activity. Another issue left still unresolved is the relative capacity for the cleavage enzyme(s) for carotenoids other than p-carotene. Despite the use of crude enzyme preparations to establish relative specificities of the cleavage activity for various carotenoids, in *vivo* studies to test the biopotency of the various carotenoids have not yielded data that correlate well with the in *vitro* observations (Singh and Cama, 1975).

What is widely accepted, however, are two important conclusions. The first is that the carotenoids cleaved to retinal will be reduced to retinol and be esterified with palmitate for extra-intestinal transport. The second conclusion is that in man and many animals, large quantities of intact carotenoids are absorbed via the intestinal mucosa. Recent work has focused on dietary factors that might influence either the cleavage process or the absorption of p-carotene.

Several dietary factors have been recently examined for possible influence on carotenoid bioavailability, including alcohol, fiber, protein, and fat content of diets. Using a chronic alcohol consumption rat model, Grummer and Erdman (1983) demonstrated that rats fed ethanol as 30% of their caloric intake, absorbed as much p-carotene as control rats not consuming alcohol. Despite finding alterations in the extra-intestinal metabolism of vitamin A, their data suggests that ethanol does not influence the bioavailability of p-carotene. Erdman et al. (1986) showed that in chicks fiber consumption will alter p-carotene utilization. Specifically, 7% dietary hemi-cellulose, lignin and apple or citrus pectin reduced p-carotene bioavailability as evidenced by decreased liver vitamin A stores compared to controls. The authors speculate that the high viscosity of the gastrointestinal contents due to these fibers resulted in phase separation of bile from fat-soluble materials, therefore disrupting micelle formation. The work of Wahid (1985) suggests that male rats consuming a low protein diet of 5% casein will sub-optimally metabolize and absorb p-carotene. Interestingly, a high quality fish protein diet enhanced p-carotene absorption compared to casein protein consumption when both constituted 20% of the diet. A recent investigation using human subjects concluded that dietary fat level influences p-carotene bioavailability (Dimitrov et al., 1988). A low-fat diet consumed by healthy human subjects for five days resulted in lower plasma levels of p-carotene than those of high-fat diet consumers that were also consuming 45 mg of p-carotene per day.

The relationship of p-carotene intake to tissue concentrations has been investigated in both man and animals. The most extensive investigation to establish this relationship in rats was recently published by Shapiro et al. (1984). Female rats fed a series of levels of p-carotene ranging from 0.002% to 0.2% of diet for 21 weeks were subjected to post-mortem tissue analysis for p-carotene content. Interestingly, when the various tissues, including liver, adrenal, ovary, lung, heart,

kidney, plasma, skin, brain and muscle were compared, there was a very wide range of concentrations and differences in dose-response curves. The liver was revealed to be the major site of storage, while tissues feuch as muscle and brain had very low levels of accumulation. In man, a study by Parker (1988) confirmed several earlier reports that adipose tissue was a major site of carotenoid accumulation. Analyzing abdominal adipose tissue from 19 adults, Parker concluded that p-carotene and lycopene were the predominant carotenoids. An estimate of mean total adipose carotenoid concentration suggested that the levels in adipose were similar to those of the liver in published reports and that together would account for most of the carotene in the human body.

Other investigations have more thoroughly assessed the relationship of carotenoid consumption with serum carotenoid levels (Willett et al., 1983; Katrangi et al., 1984; Tangney, 1984; Jensen et al., 1985; and Kim et al., 1988). Our recent study of the effects of carrot juice consumption upon serum a- and p-carotene and total carotenoid levels illustrates the complexities of relating specific dietary carotenoids to their appearance in serum (Kim et al., 1988). Five adult human subjects consumed sixteen ounces of carrot juice per day, providing approximately 40 mg of carotenoids composed of 20 mg p-carotene, 10 mg a-carotene and 10 mg of other carotenoids, for seven days and then eight ounces every other day for the next seven days. Another group of five subjects consumed sixteen ounces of juice per day for seven days and then received no carrot juice for *the* next seven days. Blood serum collected from both groups at the start of the study and after day seven and day fourteen was analyzed by HPLC and spectrophotometry for carotenoid concentration. When consumption of carrot juice was continuous, Q-carotene and total carotenoids increased at one week and stabilized, while p-carotene continued to increase over the course of the two weeks. When carrot juice consumption occurred for one week and was then terminated, a- and p-carotene rose at one week and then stabilized, while total carotenoids rose at one week and then returned to baseline level after the week of withdrawal.

Other researchers have shown that a daily p-carotene supplement of 30 mg for eight weeks approximately tripled total plasma carotenoid levels (Willett et al., 1983). A study by Tangney (1984) showed that 30 or 60 mg of p-carotene per day increased serum p-carotene levels up to four times of baseline levels by day 16. In other studies, serum a- and p-carotene levels have been reported to double with carotenoid consumption, with small or non-significant increases in total serum carotenoid concentration (Katrangi et al., 1984). Jensen et al., (1985), feeding subjects whole carrots providing about 40 mg of carotenoids per day primarily as equal amounts of a- and p-carotene, demonstrated that a- and p-carotene) increased equally in blood after consumption.

Only one report has been presented that suggests a non-dietary influence upon tissue carotenoid levels (White et al., 1988). Significant reductions in plasma total carotenoid levels were seen in male and female human subjects exposed repeatedly to ultraviolet irradiation. The researchers speculate that the reductions may have resulted from either a direct photodecomposition or photo-isomerization of carotenoids, or indirect photodecomposition of carotenoids due to interactions with expanded population of reactive species such as peroxyradicals resulting from the ultraviolet irradiation.

The importance of all of these studies is elevated by numerous recent observations of the inverse relationship of carotenoid consumption to the development of various types of cancers. A recent review by Temple and Basu (1988) concludes that the existing data indicate that p-carotene protects against lung cancer and probably stomach cancer. Other possible sites include ovary, cervix and breast. Some recent animal feeding trials indicate that some carotenoids may function as anti-oxidants in *vivo* (Blakely et al., 1988), while other studies show that carotenoids may function as enhancing agents for the immune system (Bendich and Shapiro, 1986).

4. Analysis

The usual carotenoid analysis consists of extraction of the pigments from plant tissue, partial purification by saponification, separation into three classes according to the number of hydroxyl groups, isolation by chromatography, identification and measurements by spectral absorption, and confirmation of identification by special methods. Isolation procedures include open column, thin-layer, and high performance liquid chromatography (HPLC). Due to the tediousness and complexity of these time consuming procedures, one or more of these steps may be eliminated or modified depending on the nature of the system under study and the amount of information required.

In the typical AOAC procedure, the sample is not saponified prior to chromatography on a MgO column. The column is developed with a mixture of petroleum ether and acetone. The assumption is made that the first fraction to elute is p-carotene and that the xanthophylls are retained on the column. In practice the values are good for green vegetables where p-carotene is the major carotene, and the oxygenated xanthophylls which are not precursors of retinol are retained. The method is also good for vegetables like butternut squash or sweet potato etc. where p-carotene is the major carotenoid present. a-Carotene which is found in large amounts in the carrot is not well separated by the AOAC method, typically a-carotene chromatography with p-carotene.

In the spectrophotometric analysis both a- and p-carotene absorb light at similar wavelengths, resulting in overestimation. In many fruits the xanthophylls are esterified and are thus less polar; hence they may chromatograph with p-carotene. In the case of the

dihydroxy, the overestimation could be large. In the case where p-cryptoxanthin (a vitamin A precursor) is esterfied, the overestimation is less. By the use of a gradient elution, complex mixtures can be resolved and saponification can be used on certain tissues. Table 3 shows data for these various possibilities where fruits and vegetables are divided into three groups. Kang-Kong is a green leafy vegetable common to South East Asia.

Table 3. Provitamin A activity of selected vegetables and fruits (mg p-carotene **equiv./100 gm)**

	Group 1		Group 2		Group 3	
Analysis method	Kang-kong	Spinach	Carrot	Pumpkin	Loquat	Papaya
AOAC method	3.27	2.06	13.39	10.03	2.19	1.79
Stepwise solvent gradient elution	–	–	10.58	4.84	–	–
Saponification & stepwise solvent gradient elution	–	–	–	–	1.20	0.59
HPLC method	3.32	1.99	10.83	5.09	1.04	0.52
Major carotenoids	β-carotene		α-carotene β-carotene		β-carotene β-cryptoxanthin	

From Simpson and Tsou (1986).

During the last ten years with the advent of HPLC, numerous analytical methods have been published for the separation of individual provitamin A carotenoids. HPLC techniques are considered to be the simplest and most reproducible methods of analyzing carotenoid mixtures in foods. Sweeney and Marsh (1970) and Stewart (1977) separated the prevalent provitamin A carotenoids in fruits and vegetables using non-commercial columns. Since then numerous commercial columns of both normal and reversed-phase have been used with and without gradients (Zakaria et a!., 1979; Bushway and Wilson, 1982; Bushway, 1985; Quackenbush, 1987). However, no single method thus published can be used universally for all vegetables due to the inability of separation of all provitamin A carotenoids and their isomers.

For a more in depth treatment of the AOAC method as well as TLC, gradient elution and HPLC, the paper by Simpson et al., (1985) may be consulted.

5. Effect of Postharvest Storage on Provitamin A Carotenoids

There is a limited number of reports on the carotenoid changes during post-harvest storage. Takama and Saito (1974) reported the effect of storage on total carotenoid in sweet pepper and parsley at two different temperatures. At cold room storage (7° C), both sweet pepper and parsley lost over 20 % of their total carotenoids, while at room temperature (15-17° C), sweet pepper and parsley lost 60% and 80%, respectively, during 9-day storage (Fig. 1). The carotenoids in kale and collards also appear to be unstable during storage. Ezell and Wilcox (1962) reported that kale and collards stored in open paraffin cardboard containers at low temperature (0° C) for four days lost less than 10% of total carotenoids, but when stored at 10°C and 21° C they lost over 22% and 65%, respectively (Fig. 2). These losses are significant and it appears that storage temperature and time have a great influence on carotenoid stability in fresh green and leafy vegetables.

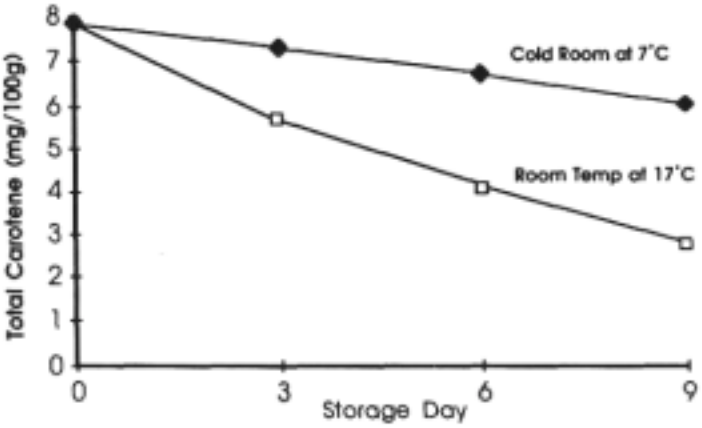
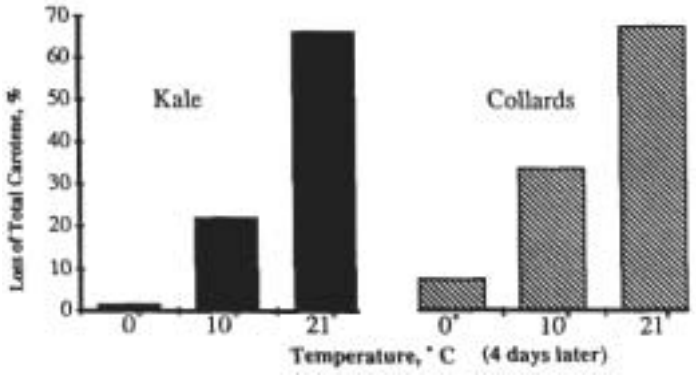


Figure 1. Total Carotene Content of Sweet Pepper During Storage (Takame & Saito, 1974)

Figure 2. Effect of Storage Temperature on Carotene in Fresh Vegetables (Ezell & Wilcox, 1962)



On the other hand, the amount of carotene in other vegetables such as winter squash, sweet potatoes and carrots has been reported to increase during their storage (Brown,1949; Rygg, 1949; Ezell et al., 1956; Lee, 1986). Hopp et al., (1960) reported that the p-carotene content in three cultivars of winter squash increased slowly during a storage of 25 weeks at temperatures ranging from 23° to 9° C and relative humidities (RH) from 57% to 68 % (Fig. 3). Sweet potatoes stored for six months at 15° C and 75% to 95% RH showed little increase in total carotenoid (Ezell et al., 1956). Lee (1986) has reported changes in individual provitamin A carotenes in carrots during storage: the Nantes cultivar stored in open containers at 2° C and 90% RH for 155 days showed an increase in all four

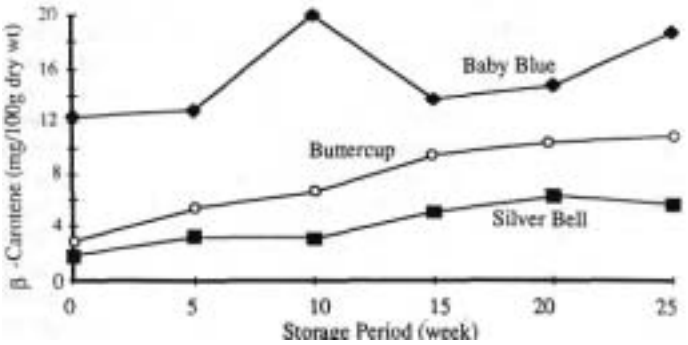


Figure 3. Carotene in Winter Squashes During Storage From Hopp et al, (1960)

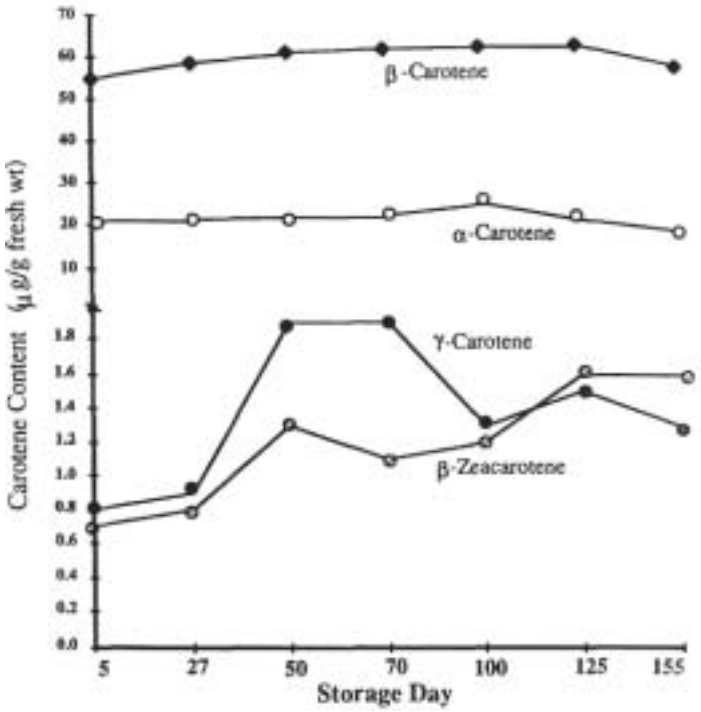


Figure 4. Change in Provitamin A Carotenes in Carrots During Storage (Lee, 1986)

provitamin A carotenes; a-, (}-, y-carotene and p-zeacarotene. They increased gradually during storage up to 100-120 days, and then leveled off slowly (Fig. 4). These increases contrast greatly to those of fresh green vegetables, which had showed a drastic decrease during storage. Therefore, we can state that the changes in the carotene content of vegetables during post-harvest storage are significant but depend upon the crop and the storage conditions.

Another important criterion in the evaluation of the carotenoid content of fruits and vegetables is related to maturity. We have found that younger carrots contain much less carotene than old or mature carrots (Lee, ,1986). Therefore, if one compares the carotenoid /content of young fresh carrots with the processed product made from old carrots, the large differences observed might be due mainly to the differences in maturity.

Unlike many other nutrients, the carotenoid content varies greatly among different cultivars of the same crop. Ezell et al., (1956) have shown that two cultivars of sweet potatoes differ in carotenoid content by 6 to 7 fold. Among six cultivars of winter squashes studied by Hopp et al., (1960), Sweet Meat and Silver Bell contained less than one-half the carotenoids of Butternut and less than one-seventh of the content of the Baby Blue cultivar. Similar large differences were observed in cultivars of sweet potato (Reddy and Sistrunk, 1980), sweet corn (Lee et al., 1981), and orange juice (Taylor and Witte, 1937). In addition, Bunnell et al., (1958) reported large seasonal variations in the carotenoid content of the Florida orange concentrates. Sweeney and Marsh (1971), conducted extensive research on commercially processed vegetables by analyzing individual provitamin A carotenoids and their stereoisomers. Among processed pumpkins, they found that the total carotene content of one commercial brand was about 17 times higher than that of another brand, while one commercial sweet potato brand was about 42% higher in total carotene than another (Table. 4). Therefore, a proper sampling procedure is a definite prerequisite when studying the carotenoid content of fruits and vegetables.

Table 4. Carotene Stereoisomers in Canned Foods

Isomer	Pumpkin			Sweet Potatoes	
	Brand 1	Brand 2		Brand 1	Brand 2
Total Carotene* (μg/100g)	9,880	580	(%)	4,640	6,600
Neo-α-carotene β	5.3	–		–	–
All-trans-α-carotene	35.1	3.3		–	–
Neo-α-carotene U	3.3	–		–	–
Neo-β-carotene	2.0	–		3.3	5.7
Neo-β-carotene B	6.2	20.5		10.2	6.2
All-trans-β-carotene	43.2	62.4		79.5	82.9
Neo-β-carotene U	4.2	13.8		7.0	5.2
Neo-β-carotene V	0.7	–		–	–
Effective Carotene (μg/100g)	5800	460		4150	6020

*** Based on assumption that carotene is entirely all-trans p-carotene.**
From Sweeney and Marsh, (1971)

6. Effect of Processing on Provitamin A Carotenoids

Many reports have been published on the effect of heat processing on total carotenoids. The total carotenoid content during processing may not be altered, leading to the assumption that there has been no loss, but isomerization does occur and each provitamin A carotenoid and each stereoisomer have different biopotency values (Zechmeister, 1962) as noted previously. Therefore, we have to know the distribution of the provitamin A carotenoids in order to assess with accuracy the vitamin A value of a food. The work of Gebhardt et al., (1977) on peaches clearly shows the difference between two analytical methods on the apparent vitamin A values. The AOAC method gave about 300% more in vitamin A in fresh peaches than the other chromatographic method that analyzed individual provitamin A carotenoids. When the biological

effectiveness of each stereoisomer was taken into account, the vitamin A value decreased further, from 456 to 321 mg/100g(Table5).

The results from our laboratory (Edwards and Lee, 1986) and others (Weckel et al., 1962; Baloch et al., 1977) have shown that the loss of soluble solids must be taken into account in calculations in order to obtain accurate carotenoid values in processed carrots. Many reports have shown higher carotenoid concentrations in canned carrots when the results were expressed in dry weight. This is mainly due to a leaching of soluble solids into the canning liquid and calculations based on total carotenoid (Table 6). Baloch et al., (1977) have shown the magnitude of carotene increase on dry weight due to the loss of soluble solids (Table 7). When we analyzed the carotenes of green peas, results similar to canned carrots were obtained in that the canned product showed the higher value based on dry weight . However, when we included loss of soluble solids by analyzing the entire content of the can, the overall result was lowered (Edwards and Lee, 1986). A similar result was obtained by other workers (Deudek et al., 1982).

In a recent study on the effect of processing on the carotenoids of vegetables, we found that the enzymatic systems in the vegetable oxidize carotenoids significantly during analysis. Since there have been many reports that some chlorophyll-containing vegetables possess enzymatic systems, mainly lipoxygenase and peroxidase, that oxidize carotenoids

Table 5. Comparison of Analytical Methods of Carotene in Peaches

Method	mg/100g Raw	mg/100g Canned	Retention (%)
AOAC method	1790	552	31
Provitamin A carotenoids	456	386	85
Effective carotene	321	270	84

From Gebhardt et al., (1977)

Table 6. Carotene Content of Fresh and Canned Carrots

	Carrot	Fresh	Canned	
			Drained	Non-drained
			(µg/g d.w.)	
Total		1,331	1,338	929
	(I.U./g)	2,218	2,230	1,549
	α-Carotene	368	456	302
	β-Carotene	800	843	531
	β-Zeacarotene	17	12	8
	γ-Carotene	15	13	10
	Total	1,190	1,324	851

From Edwards and Lee (1986)

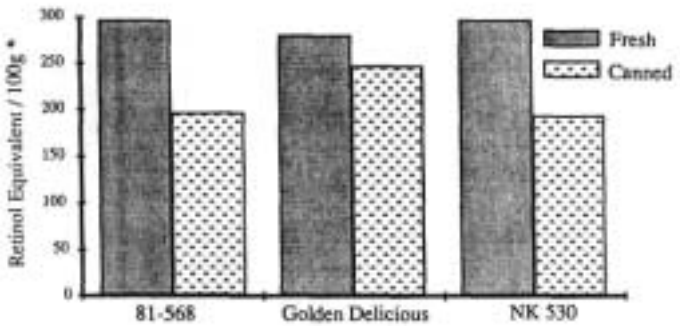
Table 7. Effect of Leaching of Soluble Solids on Carotenoid Content of Processed Carrot

Treatment	Loss in soluble solids (% d.w.)	Increase in carotenoid (% d.w.)
Unblanched	--	--
Blanched	2.7	9.1
Water dipped	6.9	26.1
Detergent dipped	7.5	29.1
Water washed	11.9	48.2
Detergent washed	14.5	59.0

(Booth, 1960; Ueno et al., 1982; Ben Aziz et al., 1971; Lee and Smith, 1988), we added an antioxidant, pyrogallol, to fresh peas before the grinding and extraction, and then found a higher vitamin A value. This indicated that enzymatic destruction of carotenoids was responsible for the lower carotenoid content in fresh peas. When a large quantity of peas (100-200 g) was taken to isolate individual provitamin A carotenes, it took at least two hours for grinding, filtration, and extraction; this gave the enzymes enough time to activate and bleach the carotenoids. In a study of fresh ground peas, we found a 68% decrease in total carotenoids in two hours if the slurry was left at room temperature, while the pyrogallol treated peas lost a minimal amount (Edwards and Lee, 1986). We concluded from this that the apparent increase in carotenes in processed peas is not an actual increase, but rather, it is due to the enzymatic destruction of carotene in the fresh peas during analysis.

Sweeney and March(1971) reported on the effect of cooking on carotenoids in vegetables. They have shown that heating promotes *cis-trans* isomerization by decreasing the *a\\-trans* carotenes and increasing their c/s-isomers. Broccoli lost 13 % of *a\\-trans* P-carotene and increased c/s-isomer U by more than 80%; spinach lost 7% of *a\\-trans* and increased the c/s-isomers; sweet potatoes lost 32% of *a\\-trans* and increased c/s-isomer by 236 fold. Overall, the losses of effective carotene were 8%, 4%, and 15%, for broccoli, spinach, and sweet potatoes, respectively. Weckel et al., (1962) and our group (Ogunlesi and Lee, 1979) reported that the loss of vitamin A in canned carrots (about 15%) was mainly due to the *cis-trans* isomerization of a- and p-carotenes during canning. We also observed during canning that the provitamin A carotenoids of winter squash decreased 13%-35%, depending on the cultivars as shown in Figure 5 (Lee et al., 1984). High temperature and short time (HTST) processing appears to have less effect on *cis-trans* isomerization in sweet potatoes (Lee and Ammerman 1974). Valadon and Mummery (1981) reported the change in carotene during puree preparation and storage of oranges. They found a 43% decrease in Spanish and 15% in Turkish oranges during processing and a further decrease

Hnrinn ctnrano



* Included a -, p -,7 -carotene and p-zeacarotene.

Figure 5. Vitamin A Value of Fresh and Canned Winter Squash From Lee et al., (1984)

Dehydration and powder forms of fruits and vegetables increase surface area and lead to very poor stability of carotenoids in general, unless the products are protected from air and light. Water has a protective Effect on the autoxidation of carotenoids and, therefore, water activity is related to carotenoid stability in dehydrated products (Ramakrishan and Francis, 1979; Haralampu and Karel, 1983). Since carotene degradation is known to be a free radical process, interaction with water reduces oxidation. Goldman et al. (1983) reported that the shelf-life of carotene in the dry state was shorter than that in the wet state. Different dehydration processes have shown different retentions of *a*\\-*trans* p-carotene in carrot dices (DellaMonica and McDowell, 1965): based on fresh carrots at 100%; blanched gave 95% retention; freeze-dried, 89%; conventional air dried, 80%; and explosive puff-dried, 72%. Freeze drying appears to have only a minor effect on the carotenes of carrots (Sweeney and Marsh, 1971). Bolin and Stafford (1974) reported that sulfur dioxide stabilized carotenes in apricots during dehydration. A study on aseptic packaging and storage effects on the carotenoids of guava and papaya puree were reported by Chan and Cavaletto (1982). They showed that aseptic processing itself did not have a significant effect on total carotenoids but that the level decreased significantly during storage. Simpson (1985) reviewed the chemical changes in natural pigments including the carotenoids. This included the *cis-trans* isomerization, epoxidation and enzymatic and non-enzymatic changes in the carotenoids.

Final Remarks

Cooperative research between some of the laboratories represented in the NE-116 group has addressed the problems of the analysis of the provitamin A carotenoids. This, in turn has allowed determination of the carotenoids losses during storage, canning, freezing etc. Also, some of the methods of analysis which were developed under this association are now being used to update the nutritional tables and are being used in the NCI computer program for vitamin A determinations.

It is critical that we have accurate values in order to determine vitamin A status, as well as to extend the studies to include genetic manipulations leading to increased carotene production. Since carotene is increasingly being shown to be important in many areas of health apart from being a vitamin A source, its increased production through selection of superior breeding lines and preservation in storage and processing is important.

Many research papers dealing with post-harvest storage and processing effects on carotenoids have been published, but most of them are based on the conventional total carotene analysis. In addition, some important criteria, such as differences in cultivars, ^maturity, and proper sampling procedures were not considered. Therefore, it is hard to make conclusions based on those data. However, a few studies that have been carried out properly have indicated that:

1. Loss of carotenoids during post-harvest storage of fruits and vegetables is significant but varies with the crop and storage conditions: some leafy vegetables lose a significant amount of carotenes, the rate of loss being proportional to the temperature and time. On the other hand, sweet potatoes, carrots, and winter squashes show a slight increase in carotenoids during storage.

2. Heat processing, especially canning and high temperature drying, cause a marked reduction in carotenoid levels while freezing and other low temperature processing have less of an effect. HTST processing that minimizes destruction of *a*\\-*trans* carotene and formation of c/s-isomers appears to have a protective effect on carotenoids.

Considering the importance of today's nutritional quality of foods, we need a critical evaluation of provitamin A carotenoids in fruits and vegetables so that, ultimately, we can understand better the effects of storage and processing.

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