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Preface

The Latin American Congress on Food Carotenoids was convened on September 14-17, 1998 in Campinas, São Paulo, Brazil, with the following objectives:

1. To provide Latin American researchers with up-to-date scientific and technological information on various aspects related to carotenoids;
2. To discuss and integrate research findings on carotenoids in Latin America and stimulate practical applications of these results in the industrial-economic development and improvement of the public health status of the region;
3. To identify carotenoid-related research needs and priorities in Latin American and to stimulate collaborative work, thereby increasing the research capacity of the region.

The congress brought together 90 participants from different fields of studies, with speakers from Switzerland, England, U.S., Germany, Argentina, Chile, Mexico, Venezuela and Brazil. Research papers were also presented orally and as posters. The animated discussions that followed the presentations reflected the profound interest the different topics had drawn among the participants. The conference covered the many facets of carotenoids from the basic science to applications in health, nutrition and food science and technology. The wealth of informationn generated merits publication, hence this special supplement of the Archivos Latinoamericanos de Nutrición. Unfortunately, for one reason another, the supplement does not include the excellent lectures of Dr. Hanspeter Pfander and Dr. George Britton. Dr. Pfander talked on "Carotenoids - Colors for Life", "Carotenoid Chemistry: Synthesis, Reactions, Oxidation" and "Identification and Structure Elucidation". Dr. Britton lectured on "Current Knowledge of Carotenoid Biosynthesis" and "Relation Between Structure, Properties and Functions".

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Carotenoids and human health

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SUMMARY. After the discovery of vitamin A in 1913, the yellow pigments of fruits and vegetables were soon implicated as compounds with similar nutritional effects. ß-Carotene was shown to be converted into vitamin A by Moore in 1929, and the chemical structures of both vitamin A and ß-carotene were determined two years later. Thus, the sole function of ß-carotene in human health was considered to be its conversion into vitamin A. On the basis of observational epidemiologic studies, conducted in the mid-1970s, however, carotenoids were implicated as protective agents, first against lung cancer and then against a variety of other chronic diseases. Intervention trials employing ß-carotene, however, either have shown no preventive effect or indeed, in two cases, have enhanced the incidence of lung cancer in middle-aged male smokers and asbestos workers. The possible protective action of carotenoids can be attributed to their properties as singlet oxygen quenchers and as antioxidants, whereas their cancer-enhancing actions in lung can be ascribed to the prooxidant action of carotenoid free radicals in damaged cells. Apart from chronic diseases, ß-carotene has shown significant therapeutic value in individuals suffering from photosensitivity disorders and provides temporary relief to persons afflicted with leukoplakia. Apart from a medical context, the colored carotenoids found in many living organisms and in many foods delight both the eye and the palate. Thus, human health and the enjoyment of life are greatly benefited by the presence of these interesting pigments in nature, whether or not they ultimately prove to have more specific protective effects against chronic diseases.

Key words: Carotenoids, human health, chronic diseases, antioxidant.

RESUMEN. Carotenoides y salud humana. Después del descubrimiento de la vitamina A en 1913, los pigmentos amarillos de frutas y vegetales fueron inmediatamente implicados como compuestos de efectos nutricionales similares. La conversión de ß-caroteno en vitamina A fue mostrada por Moore en 1929, y las estructuras químicas de la vitamina A y el ß-caroteno fueron determinadas dos años después. Así, pensábase que la única función del ß-caroteno para la salud humana sería su conversión en vitamina A. Basados en estudios de observaciones epidemiológicos, conducidos en mediados de 1970 sin embargo, los carotenoides fueron implicados como agentes protectores, primero contra el cáncer de pulmón y después contra una variedad de otras enfermedades. Ensayos de intervención utilizando ß-caroteno, no obstante, no han mostrado efecto preventivo o, en dos casos, han aumentado la incidencia del cáncer de pulmón en fumadores masculinos de mediana edad y trabajadores del asbesto. La posible acción protectora de los carotenoides puede ser atribuida a las propiedades como secuestrante de oxígeno singulete y como antioxidantes, mientras que sus acciones como promotores de cáncer en el pulmón pueden ser asignadas a la acción prooxidante de radicales libres de carotenoides en células malogradas. Además de las enfermedades crónicas, el ß-caroteno ha mostrado valor terapéutico significativo para los males de la fotosensibilidad y en individuos con leucoplaquia. Aparte del contexto médico, los carotenoides encontrados en muchos organismos vivos y en muchos alimentos agradan a los ojos y al paladar. Por lo tanto, la salud humana y el disfrutar de la vida son grandemente beneficiados por la presencia de estos interesantes pigmentos en la naturaleza, sea que finalmente se pruebe o no que tengan efectos protectores más específicos contra las enfermedades crónicas.

Palabras clave: Carotenoides, salud humana, enfermedades crónicas, antioxidante.

INTRODUCTION

Carotenoids have been known as distinct entities in nature for more than a century and a half (1). ß-Carotene, a hydrocarbon, was first crystallized from carrots in 1831 and more polar carotenoids, the xanthophylls, were isolated from autumn leaves a few years later. A large number of carotenoids in nature were identified in the early 1900s by chromatographic techniques. Some common naturally occurring carotenoids are depicted in Figure 1 (1).

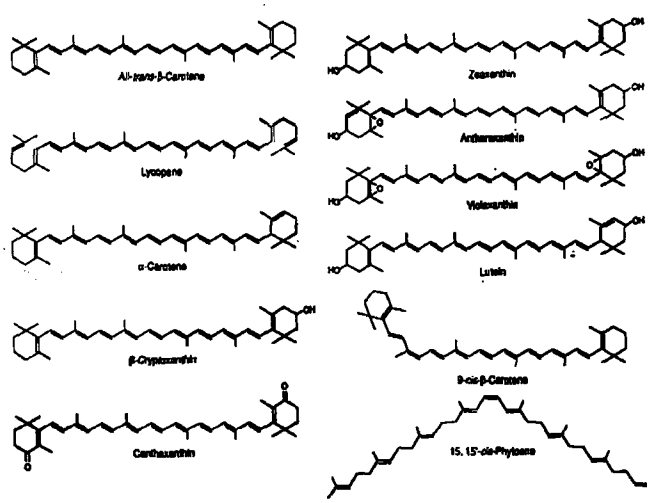
Vitamin A was discovered as a stimulant for rat growth in 1913, and some carotenoid pigments, but not all, were shown to act similarly a few years later. Various speculations concerning the possible role of carotenoids were resolved in 1929 by the demonstration that carotenoids are converted into vitamin A (2). Two years later, the chemical structures of both ß-carotene and retinol were determined by Karrer, who postulated that the addition of two molecules of water across the central bond of ß-carotene could yield vitamin A. The actual mechanism of this cleavage reaction, however, was not clarified for more than 30 years.

Carotenoids have several functions in nature. They serve as provitamin A compounds in animals and humans; they provide protective and mating coloration in birds; they serve

as ancillary light-gathering pigments in photosynthesis; and they protect chlorophyll from oxidative damage in photosynthetic organisms. They also are involved in the xanthophyll cycle in plants, by which light energy is dissipated without destroying the plant cells. In addition to these functions, carotenoids have been shown to have other actions in physiologic systems and to be associated with protection from chronic diseases.

In the 1970s, as the field of observational epidemiology grew, a dietary vitamin A index was shown to be associated with protection from lung cancer (3). It was unclear at that time whether the vitamin A index related primarily to vitamin A, primarily to provitamin A carotenoids, or to a mixture of the two. This query was resolved in large part in 1981, when dietary carotenoids, but not vitamin A, was shown to be associated with cancer chemoprevention (4). Since that time, a large number of studies have been conducted germane to the possible roles of carotenoids in the prevention of chronic diseases.

FIGURE 1
Some common naturally occurring carotenoids (1)



Therapeutic benefits

Photosynthetic disorders: Certain individuals with genetic defects in porphyrin metabolism are extraordinarily sensitive to sunlight (5). Some common conditions of this kind include erythropoietic protoporphyria, congenital porphyria and polymorphic light eruption. In erythropoietic protoporphyria the enzyme ferrochetolase is defective. As a consequence, iron is poorly incorporated into the protoporphyrin ring to yield heme, a key component of hemoglobin, the cytochromes and other heme-containing enzymes. As a consequence, free protoporphyrin circulates in the blood and is taken up by the skin, where it absorbs light and ultimately forms singlet oxygen. This highly reactive form of oxygen interacts with many components of cells, killing them and causing skin lesions.

When large amounts of ß-carotene are administered daily to patients with these disorders, the ß-carotene also accumulates in the skin, quenches the singlet oxygen, and minimizes tissue damage. Doses of 180 mg of ß-carotene, of which only a fraction is absorbed, have been ingested daily by individuals with these disorders to good effect. Interestingly, these huge doses do not cause vitamin A toxicity, primarily because of the relatively slow conversion of ß-carotene into vitamin A in the body. Nonetheless, they do cause a yellowing of the skin, termed hypercarotenosis, which is, of course, at the basis of its therapeutic utility.

At the termination of dosing, the carotenoids are slowly cleared from tissues, and the sensitivity to light returns. Thus, ß-carotene does not cure but only ameliorates the condition as long as it is present.

Human leukoplakia: Cancer of the oral cavity is a common malignancy in many parts of the world (6). Leukoplakia, namely a white patch or plaque on the buccal mucosa that cannot be rubbed off, is considered to be a pre-malignant lesion. When relatively large doses (30-180 mg) of ß-carotene are administered daily to humans for 3-9 months, 40-60% of the subjects respond positively as compared to 10-20% of patients treated with a placebo (6). Large doses (400 IU) of vitamin E show a similar effect. Upon termination of treatment, however, the lesions return. Thus, this response also tends to be phenotypic rather than curative. Supplements of ß-carotene have not been shown to decrease the incidence of cancer of the oral cavity.

Cancer

Carotenoid intakes, including supplements, have been implicated as protective factors against a wide variety of human cancers (7-11) (Table 1).

TABLE 1
Organs that may be protected against cancer by carotenoids

Lung
Oral cavity, pharynx and larynx
Esophagus and stomach
Colon and rectum
Breast
Prostate
Cervix
Skin

Lung: Lung cancer is the leading cause of cancer death in men and women in the United States. Heavy smoking is by far the dominant controllable risk factor. Diet also seems to be important. A large number of observational epidemiologic studies, namely 8 of 8 prospective studies and 18 of 20 retrospective studies, for example, showed a significant association between the intake of carotenoid-containing

vegetables and fruits and a reduced lung cancer risk (7-10). These findings stimulated the conduct of a large clinical trial in Finland in which α-tocopherol and ß-carotene were used as supplements (12). In this study, 29,133 Finnish male smokers, aged 50-69 years, were divided into four groups; namely, a ß-carotene supplement group, an α-tocopherol supplement group, a ß-carotene plus α-tocopherol group, and a placebo group. Treated groups received 20 mg of ß-carotene and/or 50 mg of α-tocopherol daily for 5-8 years. The results of the study were highly unexpected. Rather than preventing lung cancer, ß-carotene enhanced lung cancer incidence by 18% (95% confidence interval, 1.03-1.36) and death by 8% (95% confidence interval, 1.01-1.16). α-Tocopherol had no effect on lung cancer incidence, either in a positive or negative fashion.

The findings in the Finnish study were confirmed in a large similar study conducted in the northwestern United States (13). In this case, 14,254 American men and women smokers, 50-69 years, plus 4,060 male asbestos workers either were supplemented with 30 mg ß-carotene plus 25,000 IU vitamin A daily or received a placebo. The study, which originally was scheduled to continue for 5.5 years, was stopped at 3.7 years because of the outcome. Supplements of ß-carotene and vitamin A enhanced lung cancer by 28% (95% confidence interval, 1.04-1.57) and mortality by 17% (95% confidence interval, 1.03-1.33).

In another major intervention trial, the Physicians Health Study (14), 22,071 U.S. male physicians, 40-84 years of age, were given 50 mg ß-carotene or a placebo on alternate days for 12 years. In the initial five years of the study, 325 mg of aspirin was also provided on alternate days. In this study, ß-carotene had no effect on the incidence of lung cancer or of total neoplasms (relative risk = 0.98, 95% confidence interval, 0.91-1.06). Only 11% of the subjects in this study, however, were current smokers. In a smaller study, 755 asbestos workers in Tyler, TX, were given a supplement of 50 mg ß-carotene plus 25,000 IU retinyl acetate every other day for five years. No differences were noted between control and treated groups in the prevalence of sputum atypia (8, 10).

Thus, a dichotomy exists. While evidence obtained from observational epidemiology supports a protective role of dietary carotenoids against cancer risk, intervention studies do not (Table 2). Some possible reasons for these differences between the outcomes of intervention trials and observational epidemiologic studies are that: (a) observational epidemiology focuses on foods that contain many components, whereas intervention trials employ single compounds, (b) the amount of a carotenoid ingested is small in dietary studies but large in intervention trials, (c) the physiologic effects of supplements may differ from those of the same nutrient in foods, and (d) utilization of other protective components of foods may be inhibited by large doses of ß-carotene. Whatever the explanation, the universal lack of a protective effect of ß-carotene supplements in cancer trials, as well as the enhancement of lung cancer found in two major trials, has discouraged the initiation of further studies of this kind.

TABLE 2
Effects of ß-carotene supplements on some cancers

Site	N	Dose	Duration	RR*	95% CI
Esophagus	3,318	15 mg	6 Y	0.96	0.78-1.18
Stomach	3,318	15 mg	6 Y	1.18	0.76-1.85
Colon/rectum	864	25 mg	5 Y	1.01	0.85-1.20
Skin	1,805	50 mg	5 Y	1.05	0.91-1.22
Prostate	29,133	20 mg	5-8 Y	1.23	0.96-1.59

*Relative risk is the ratio of cancer incidence in the cited organ of the ß-carotene group relative to that in the placebo group.

Mechanisms

Carotenoids might well be protective against cancer and other chronic diseases by a number of known mechanisms (8-10) (Table 3). For example, carotenoids quench singlet oxygen, which is a highly reactive form of the oxygen atom. Carotenoids can also scavenge peroxyl radicals and can modulate the metabolism of carcinogens. Cell proliferation is inhibited and cell differentiation enhanced by carotenoids, either directly or via their conversion to retinoids. Both vitamin A and several carotenoids stimulate cell-to-cell communication and, similarly, enhance the immune response.

TABLE 3
Possible protective mechanisms of carotenoids against chronic diseases

- Quenching of singlet oxygen
- Scavenging of peroxyl radicals
- Modulation of carcinogen metabolism
- Inhibition of cell proliferation
- Enhancement of cell differentiation via retinoids
- Stimulation of cell-to-cell communication
- Enhancement of the immune response
- Filtering of blue light

The unexpected finding that ß-carotene enhances lung cancer in heavy smokers and asbestos workers also requires explanation. Some possibilities are given in Table 4. First of all, a tissue must be damaged in order for the enhancement of cancer to occur. Thus, primarily lung cancer, of many known cancers, has been enhanced by supplementation with ß-carotene. In the presence of the free radicals of cigarette smoke and relatively high oxygen tensions, ß-carotene can form peroxides and free radicals that can enhance tissue damage. When a different organ, liver, is damaged by alcohol or carbon tetrachloride, both vitamin A and ß-carotene also can enhance hepatotoxicity and the risk of liver cancer (15).

TABLE 4
Possible mechanisms by which ß-carotene enhances lung cancer

- Concurrent exposure to ß-carotene and either cigarette smoke or asbestos fibers is essential
- Risk primarily relates to the lung
- Smoke contains many free radicals
- Lung oxygen pressures are high
- ß-Carotene can form peroxides and free-radicals that can enhance tissue damage
- Cells already damaged by components in smoke may be highly susceptible to mutations

Other possible mechanisms exist, however, to explain the enhancement of lung cancer by ß-carotene (9). ß-Carotene might inhibit the absorption of other potentially protective dietary components, such as lutein, canthaxanthin or α-carotene. Smoke might also activate macrophages to secrete oxidizing agents, which then might form ß-carotene-free radicals. Finally, ß-carotene might increase the survival of neoplastic cells by inhibiting their apoptosis. None of these mechanisms, of course, is mutually exclusive.

Other chronic diseases

A number of other chronic diseases may well be affected by carotenoid ingestion, namely, cardiovascular disease, age-related macular degeneration, cataracts and HIV infections.

Cardiovascular disease: The results of studies relating carotenoid intake or supplementation to cardiovascular disease are mixed (8-10). In a variety of studies, some have shown a protective effect, some no effect at all, and yet others an enhancing effect, albeit nonsignificant. Mechanistically, carotenoids may play a role in reducing the oxidation of low-density lipoproteins, which seem to play a key role in atherogenesis. On the other hand, carotenoids can also serve as prooxidants under appropriate conditions.

Age-related macular degeneration: Age-related macular degeneration is a major cause of blindness among the elderly (16). Major risk factors tend to be smoking, age and gender, with females having a higher incidence. Many nutritionally related risk factors exist, including lower intakes of vitamin A and zinc and lower concentrations of glutathione and ascorbic acid in eye tissue. Interestingly, the macula of the eye primarily contains only two carotenoids, lutein and zeaxanthin, which are distributed in a very specific pattern within that organ. Markedly increased intakes of lutein and zeaxanthin increase blood concentrations many-fold but have a much smaller effect on the deposition of macular pigments. Mechanistically, lutein and zeaxanthin can serve as filters of blue light, thereby protecting the retina, or might serve as antioxidants. Thus far, however, intakes of lutein and zeaxanthin have not been convincingly shown to protect against this disease.

Cataracts: Cataracts consist of a gradual opacification of the lens with aging, which may in part result from oxidative stress (7-10). Carotenoid intake, as well as that of vitamins C and E, has been associated with a reduced risk of cataract. However, supplements of ß-carotene, selenium and α-tocopherol were not associated with protection against cataracts. Thus, data supporting a role for carotenoids as protective agents against cataract is currently inconclusive.

HIV infections: In HIV infections, T-helper cells are destroyed, thereby impairing the immune response (10,17). In humans, both ß-carotene and canthaxanthin enhance the immune response. Indeed, large doses of ß-carotene have been shown to increase the CD4:CD8 ratio, which is usually depressed in HIV infections. Thus, in treating this disorder, ß-carotene seems to improve the immune response and thereby decrease the incidence of infections characteristic of the disease (10,17).

Other carotenoids

Other carotenoids have also been associated with beneficial effects on human health; namely, α-carotene in lung cancer (9), lycopene in prostate cancer (11), and lutein and zeaxanthin, as already mentioned, in age-related macular degeneration (16). In no case, however, has conclusive evidence been presented that carotenoid supplements will substantially protect against any of these chronic diseases.

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REFERENCES

1. Olson JA, Krinsky NI. The colorful, fascinating world of the carotenoids: important physiologic modulators. FASEB J 1995;9:1547-50.
2. Moore T. Vitamin A. Amsterdam: Elsevier, 1957.
3. Bjelke E. Dietary vitamin A and human lung cancer. Int J Cancer 1975;15: 561-5.
4. Shekelle RB, Lepper M, Liu S, et al. Dietary vitamin A and risk of cancer in the Western Electric study. Lancet, 1981;2:1185-90.
5. Mathews-Roth MM. Carotenoids in erythropoietic porphyria and other photosensitivity diseases. Ann NY Acad Sci 1993; 691:127-38.
6. Garewal HS. Carotenoids in oral cancer prevention. Ann NY Acad Sci 1993; 691:139-47.

7. Krinsky NI, Sies H, editors. Antioxidant vitamins and ß-carotene in disease prevention. Am J Clin Nutr 1995; 62:1299S-540S.
8. Mayne ST. ß-Carotene, carotenoids, and disease prevention in humans. FASEB J 1996;10: 690-701.
9. IARC Handbook of Cancer Prevention. Vol. 2. Carotenoids. Lyon: International Agency for Research on Cancer Press, 1998.
10. Olson JA. Carotenoids. In: Shils ME, Olson JA, Shike M, Ross AC, editors. Modern nutrition in health and disease, 9th ed. Baltimore: Williams & Wilkins, 1999: 525-41.
11. Weisburger JH, editor. International symposium on lycopene and tomato products in disease prevention. Proceed Soc Exp Biol Med 1998; 218:93-143.
12. Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. New Engl J Med 1994; 330: 1029-35.
13. Omenn GS, Goodman GE, Thornquist MD, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. New Engl J Med 1996; 334: 1150-5.
14. Hennekens CH, Buring JE, Manson JE, et al. Lack of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. New Engl J Med 1996; 334: 1145-9.
15. Leo MA, Lieber CS. Alcohol, vitamin A, and ß-carotene: adverse interactions, including hepatotoxicity and carcinogenicity. Am J Clin Nutr 1999; 69: 1071-85.
16. Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am J Clin Nutr 1995; 62: 1448S-61S.
17. Santamaria L, editor. Overview and status of HIV. Int J Med Biol Environ 1995; 23: 163-282.

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Lycopene entrapped in human albumin protects 2'- deoxyguanosine against singlet oxygen damage

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SUMMARY. The generation of electronically excited molecular oxygen $^1O_2$ has been shown to occur in several biological systems, such as photooxidation of a variety of biological compounds and xenobiotics ("photodynamic action") and also enzymatic reactions. The high reactivity of $^1O_2$ with unsaturated compounds, sulfides and amino groups arises from its electrophilicity and relatively long lifetime. Thus, biological targets for $^1O_2$ having the above functional groups include unsaturated fatty acids, proteins, enzymes and DNA. There is interest in the role of nutrition in the prevention and pathogenesis of cancer. Epidemiological studies in humans have suggested that carotenoids aid in cancer prevention. Lycopene and oxycarotenoids are present at significant levels in cells and plasma. Extensively conjugated biomolecules such as carotenoids act largely on physical quenching of $^1O_2$ and in much lesser extent on chemical reaction. In this study we observed the protective effect of ß-carotene and lycopene entrapped in human albumin (HSA) against the oxidative $^1O_2$ attack of 2'-deoxyguanosine (dGuo). Photosensitization with methylene blue associated with Chelex® resine or Polymer-Rose bengal (Sensitox®) and thermodecomposition of water-soluble endoperoxide 3,3'-(1,4-naphthylidene) dipropionate were employed to generate $^1O_2$. The detection of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) and 4-hydroxy-8-oxo-7,8-dihydro-2'-deoxyguanosine (4-OH-8-oxodGuo) were performed using reversed phase HPLC with UV, electrochemical detection and by electrospray ionization mass spectrometry. Results showed a significant decrease in the amount of 8-oxodGuo in the presence of lycopene. The percentages of 4-OH-8-oxodGuo and 8-oxodGuo measured were 50% and 70% lower than the control, respectively. These data indicate that carotenoids entrapped in albumin can be an efficient quencher of $^1O_2$ and may be of interest in protecting against the deleterious effect of this excited state molecule.

Key words: Singlet oxygen, carotenoids, lycopene, 2'-deoxyguanosine, DNA, mass spectrometry.

RESUMEM. Lycopeno "atrapado" en la albúmina humana protege el 2'- deoxyguanosina contra el daño de singletes de oxígeno. La generación de singletes de oxígeno molecular ($^1O_2$) ha sido mostrada en sistemas biológicos, tales como en la fotooxidación de una variedad de compuestos biológicos y xenobióticos ("acción fotodinámica") y también en reacciones enzimáticas. La alta reactividad que presenta el $^1O_2$ con compuestos no saturados, sulfuros y grupos aminos es debido a su electrofilicidad y tiempos de vida relativamente altos. Así, compuestos biológicos que contengan estos grupos funcionales que incluyan ácidos grasos, proteínas, enzimas y DNA interactuan con singletes de oxígeno. Es interesante hacer notar el rol de la nutrición en la prevención y patogénesis del cáncer. Estudios epidemiológicos en humanos han sugerido que hay un importante rol de los carotenoides en la prevención del cáncer. Compuestos como lipoproteínas y oxicarotenoides están presentes en niveles significantes en las células y el plasma. Biomoléculas que presentan dobles enlaces conjugados extensos como los carotenoides, actúan eficientemente como inhibidores físicos del $^1O_2$ y son mucho menos eficientes químicamente. En este estudio hemos observado el efecto protector del ß-caroteno y licopeno, "atrapados" en albúmina humana (HSA), al ataque oxidativo de $^1O_2$ a 2'-deoxiguanosina (dGuo). Para generar singletes de oxígeno hemos usado la fotosensibilización con azul de metileno asociado con resina Chelex®, o bien, rosa de bengala-polímero (Sensitox®) y la termodescomposición del endoperóxido soluble en agua 3,3'-(1,4-naftilideno) dipropionato. Se registró la detección de 8-oxo-7,8-dihidro-2'-deoxiguanosina (8-oxodGuo) y 4-hidroxi-8-oxo-7,8-dihidro-2'-deoxiguanosine (4-OH-8-oxodGuo) por medio de cromatografía HPLC en fase reversa, con detección UV electroquímica y "electrospray ionization mass spectrometry". Los resultados muestran una significante reducción de la cantidad de 8-oxodGuo en presencia de licopeno. Los porcentajes de 4-OH-8-oxodGuo y 8-oxodGuo fueron 50% y 70% menores que los controles, respectivamente. Estos datos indican que los carotenoides "atrapados" en albúmina pueden ser eficientes inhibidores del $^1O_2$, y de esta manera ejercer un efecto de protección frente al deterioro capaz de producir esta molécula en estado excitado.

Palabras clave: Singletes de oxígeno, carotenoides, licopeno, 2'-deoxiguanosina, DNA, espectrometria de masa.

INTRODUCTION

The higher occupied electronic level of molecular oxygen is constituted of two π^* orbital of the same energy, so-called degenerated, occupied by only two electrons. In ground state, each of these electrons lies in one π^* orbital and their spins are parallel; hence it is a triplet state $^3\Sigma^-_g$, noted $^3O_2$ (Figure 1). As a consequence, the direct oxygen reduction by two electrons

is spin-forbidden. The activation of oxygen to electronically excited states with antiparallel spin requires overcoming of spin restriction. The first excited singlet state, $^1\Delta_g$, has two electrons with opposite spins in the same π^* orbital. The next higher excited singlet state, $^1\Sigma^+_g$, has one electron in each degenerate π^* orbital with opposite spins. The energies of the first ($^1\Delta_g$) and the second ($^1\Sigma^+_g$) singlet state of oxygen are 22.5 and 37.5 kcal/mol above the ground state, respectively (1). The second singlet state is extremely short lived (10^{-11}s), being rapidly deactivated to the $^1\Delta_g$ state and does not seem to play an important role in the oxidation processes operating in condensed phase. Thus, the term $^1O_2$ usually stands for $^1\Delta_g$ O_2.

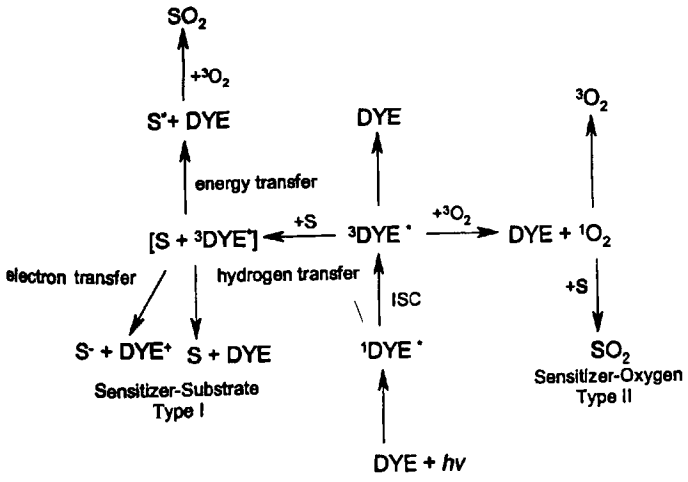
FIGURE 1

State	Orbital Occupancy	Energy (kcal/mol)	Lifetime (s)
Ground $^3\Sigma^-_g$	⟦↑ ↑⟧		
First $^1\Delta_g$	⟦↕ ○⟧	22.5	10^{-6}
Second $^1\Sigma^+_g$	⟦↑ ↓⟧	37.5	10^{-11}

Chemical and photochemical generation of singlet oxygen

Photosensitized generation of singlet oxygen: There are two fundamental types of sensitized photooxygenation (2,3). They differ in that the triplet sensitizer (DYE) reacts directly with the substrate in the Type I photoreaction, while in the second (Type II), it interacts first with oxygen to produce $^1O_2$ (Figure 2).

FIGURE 2



Type I chemistry usually involves the production of free radicals or radical-ions by interaction of the triplet sensitizer with a reducing substrate (SH or S). The produced radicals can undergo a wide variety of possible reactions such as insertion of oxygen or electron transfer to oxygen, electron or hydrogen abstraction from other substrates, initiation of free radical autoxidations, or back electron transfer reactions (3).

Gaseous discharge: The generation of $^1O_2$ by microwave discharge is well suited for studying gas phase reaction (4). The processes occurring in this system can be summarized in Scheme 1.

Scheme 1

$$^3O_2 \xrightarrow{\text{discharge}} {}^1O_2$$

$$^3O_2 \xrightarrow{\text{discharge}} 2O$$

The least desirable contaminants in this system are oxygen atoms and ozone. The main advantage of the microwave discharge method is the absence of the undesirable interactions of the substrate with an excited sensitizer or other chemical compounds, including solvent if the reaction is performed in the gas phase.

Thermal decomposition of organic ozonides: In 1969, Murray and Kaplan (5), using the triphenyl phosphite ozonide, provided experimental evidence for $^1O_2$ generation during the thermal decomposition of ozone-phosphite adducts (reaction 1).

$$(C_6H_5O)_3P\begin{smallmatrix}O\\ \diagup\ \diagdown\\ O\quad O\\ \diagdown\ \diagup\\ O\end{smallmatrix} \longrightarrow (C_6H_5O)_3P{=}O + {}^1O_2 \qquad \text{(reaction 1)}$$

The ozonide seems not to serve as a clean thermal source for $^1O_2$ at temperatures below -25°C.

Oxidation and disproportionation of superoxide anion: The possible generation of $^1O_2$ through oxidation or disproportionation of superoxide anion radical, $O_2^{\cdot-}$, led to contradictory results (6-8). Corey et al. provide evidence that $^1O_2$ *is produced through the oxidation of* $O_2^{\cdot-}$ *by various* oxidizers including Ce(IV), Pb(IV), iodobenzene and tetranitromethane in acetonitrile since the characteristic emission at 1270 nm could be recorded (9). These questions are important in biological systems since it had been recognized for a long time by Fridovich that $O_2^{\cdot-}$ is generated *in vivo* (10). The mechanism and kinetics for the oxidation of hydrogen peroxide by hypochlorite have been studied in detail (11) (reaction 2).

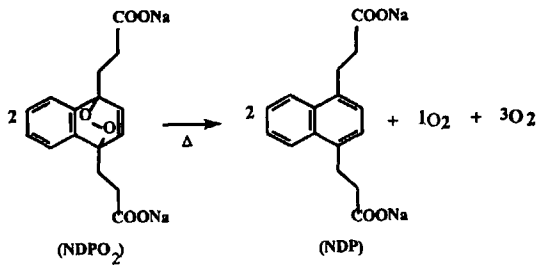
$$H_2O_2 + OCl^- \xrightarrow{HO^-} H_2O + Cl^- + {}^1O_2 \qquad \text{(reaction 2)}$$

Reaction of peroxynitrite with hydrogen peroxide: Peroxynitrite ($ONOO^-$) is a biologically active species produced

by reaction of the O_2·- with nitric oxide (NO·) (12). Nitric oxide, identified as the endothelium-derived relaxing factor (13), is formed by conversion of L-arginine to L-citrulline by NO· synthase. Endothelial cells, macrophages, neutrophils and neuronal cells have been shown to produce NO· and $ONOO^-$. Di Mascio et al. (14) propose that the peroxynitrite anion reacts with hydrogen peroxide releasing excited oxygen, once this reaction is accompanied by the characteristic chemiluminescence emission of $^1O_2$. An electronically excited intermediate of $ONOO^-$ may also be considered, *via* the reaction of the $ONOO^-$ with CO_2 (15).

Thermal decomposition of endoperoxides: The thermolysis of many endoperoxides of polycyclic aromatic compounds was suggested to occur *via* the generation of molecular oxygen and the parent aromatic species (16-19). Experimental proof for this reaction has been provided by Wasserman and Larsen (19) using 9,10-diphenylanthracene-9,10-endoperoxide. The endoperoxide is stable when stored in the solid state at 0 to 5°C, but dissociates at 80°C. The use of endoperoxide decomposition is advantageous as compared with other methods of singlet oxygen-driven oxygenations. The undesirable side-photoreactions associated with dye-photosensitization are avoided and there is no need to work at reduced pressure as in the microwave discharge methods or with low-temperature techniques as the triphenyl phosphite ozonide method. As a reproducible and clean source of $^1O_2$ for the investigation of the role of $^1O_2$ in biological systems, the use of the thermodissociation of the water-soluble endoperoxide of 3,3'-(1,4-naphthylidene) dipropionate ($NDPO_2$) is suitable. With this method, $^1O_2$ can be obtained in an easy and simple way without reactive intermediates or byproducts (reaction 3) (20). For example, this endoperoxide was used as a chemical source of $^1O_2$ to study oxidative damage to DNA (21-23).

(reaction 3)



Detection and identification of singlet oxygen

An important method for detection and characterization of $^1O_2$ is to measure the chemiluminescence arising from the radioactive transition of $^1O_2$ to the ground state. There are two types of chemiluminescence derived from $^1O_2$ (24).

Dimol emission: The bimolecular transition can be monitored by means of a red sensitive, thermoelectrically cooled photomultiplier tube connected to a discriminator, amplifier and recording system as developed by Boveris et al. (25) (reaction 4).

$^1O_2 + {}^1O_2 \rightarrow 2\ {}^3O_2 + h\nu$ (λ = 634 and 703 nm) (reaction 4)

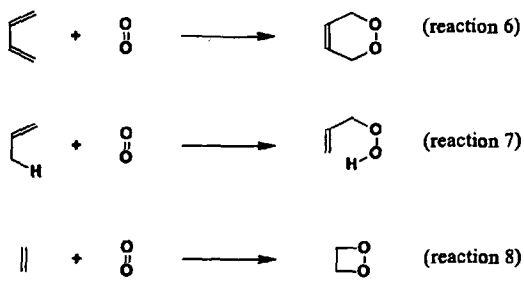
Dimol emission has often been used in complex systems such as enzymatic model reactions, suspensions of subcellular fractions and cells, perfused organ or *in situ* for an exposed organ (26).

Monomol emission: In 1979, Khan and Kasha (27) developed a spectroscopic instrumentation capable of direct solution spectral studies of $^1O_2$ emission, using a thermoelectrically cooled lead sulfite detector (reaction 5).

$^1O_2 \rightarrow {}^3O_2 + h\nu$ (λ = 1270 nm) (reaction 5)

The further development of a more sensitive spectrometer by Khan (28) based on a germanium diode photodetector and augmented the capability for examination of many reactions generating $^1O_2$. The intensity of this emission is directly proportional to the concentration of $^1O_2$, for example using the endoperoxide $NDPO_2$ (reaction 3) as a source of $^1O_2$ (20), and provides a measure of the amount produced.

Chemical traps: Trapping techniques are based on detection of the chemical product resulting from $^1O_2$ added to an appropriate substrate. The reactions of various types of substrates with $^1O_2$ are quite well established. They include the Diels-Alder reaction of dienes to form endoperoxides ([2+4] cycloaddition) (reaction 6) (29) and the "ene" reaction of alkenes to give allylic hydroperoxides (30) (reaction 7). In the $^1O_2$ "ene" reaction, olefins containing allylic hydrogens are oxidized to the corresponding allylic hydroperoxides in which the double bond is shifted to the adjacent position. In addition, $^1O_2$ reacts with electron-rich alkenes without allylic hydrogens or sterically hindered to form 1,2-dioxethanes ([2+2] cycloaddition) (reaction 8) (31).



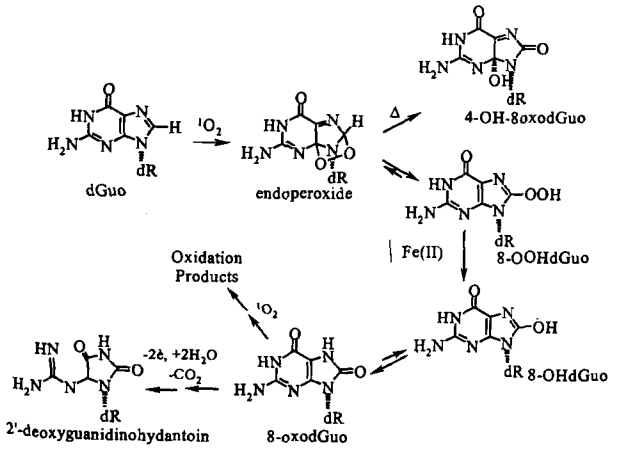
Use of deuterated solvent: The use of deuterated solvent as a tool to characterize the presence of $^1O_2$ has become universal. It is based on the remarkable fact that the lifetime of $^1O_2$ (τ) in D_2O is approximately 15-18 times longer than in water, and also longer in deuterated organic solvents (32). In cases where either $^1O_2$ and $O_2^{-\cdot}$ might be involved and the reaction is accompanied by product formation, the technique based on deuterated solvents can not be used because both $^1O_2$ and $O_2^{-\cdot}$ lifetimes are longer in those solvents (33).

Singlet oxygen in biological systems

Singlet oxygen is of substantial importance in chemical and biological systems due to its high reactivity and involvement in physiological and pathological processes. It has been shown to be generated in biological systems and implicated in (a) defense mechanisms of living organisms such as in phagocytosis, (b) hormonal activity of prostaglandins, (c) photochemotherapy utilizing the photodynamic action of synthetic dyes, (d) clinical manifestations of toxic agents like psoralens, and (e) inborn errors of metabolism exemplified by erythropoietic porphyria (34,35). The reactivity of $^1O_2$ with unsaturated compounds, sulfides and amino groups arises from its electrophilicity. Thus, biological targets for $^1O_2$ having the above functional groups include unsaturated fatty acids, proteins, enzymes and DNA. These lesions have been suggested to play an important role in aging, mutagenesis and carcinogenesis (35).

Singlet oxygen and 2'- deoxy guanosine: In DNA, $^1O_2$ reacts preferentially with 2'- deoxyguanosine (dGuo) residues, leading to the formation of at least four different reaction products: two 4R* and 4S* diasteromers of 4-hydroxy-8-oxo-7,8-dihydro-2'-deoxyguanosine (4-OH-8-oxodGuo), the main product, and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) (Scheme 2) (36,37).

Scheme 2



The formation of 4-OH-8-oxodGuo is prevented within double-stranded DNA. The reactivity of 8-oxodGuo toward $^1O_2$ is close to two orders of magnitude higher than dGuo yielding oxidation products as cyanuric acid (38). The oxidation of 8-oxodGuo via the Type I mechanism yielding 2'-deoxyguanidinohydantoin was also proposed (39).

Quenching process: Quenchers have also been employed as a means to identify $^1O_2$. Two types of quenching process may occur: chemical and/or physical (reactions 9 and 10, respectively). Chemical quenching has been already discussed as chemical trapping. Excited molecular oxygen can be physically quenched by two types of mechanism: electron transfer and energy transfer (40).

$$Q + {}^1O_2 \xrightarrow{kr} QO_2 \quad \text{(reaction 9)}$$

$$Q + {}^1O_2 \xrightarrow{kq} Q + {}^3O_2 + \text{heat} \quad \text{(reaction 10)}$$

Two classes of compounds, which apparently quench $^1O_2$ by energy transfer, are carotenoids (e.g. ß-carotene, lycopene, etc.) (41,42) and nickel complexes; in these cases the quenching rate constants ($k_t = k_r + k_q$) are *ca.* 10^{10} $M^{-1}s^{-1}$, that is slightly above those of diffusion controlled processes (43).

1,4-Diazabicyclo[2.2.2]octane, phenols, sulfides and azides are known to quench $^1O_2$ by a charge-transfer mechanism. For example, polyamines like spermine and spermidine, well known constituents of the eukaryotic chromatin, may also protect DNA against damage by $^1O_2$ (44, 45).

Carotenoids as singlet oxygen quencher

Numerous studies indicate that dietary carotenoids help reduce the risk of cancer, cardiovascular diseases, macular degeneration and cataracts (46). These are important plant pigments found in the photosynthetic pigment - protein complex of plants, photosynthetic bacteria, fungi and algae and are responsible for the bright colors of various fruits and vegetables. Several biological effects have been attributed to carotenoids. Lycopene is one of the major carotenoids in Mediterranean diets, found mostly in tomatoes and tomato products (47), and attracted attention due to its biological and physicochemical properties, especially those related to its effect as antioxidant. Color and antioxidant activities of carotenoids are a consequence of their structure, an extended system of conjugated double bonds. Carotenoids are tetraterpenes formed by tail-to tail linkage of two C-20 units, and in many carotenoids the end-groups are modified into five- or six-membered rings giving monocyclic or dicyclic compounds. Singlet oxygen quenching by carotenoids occurs via physical or chemical quenching. The efficacy of physical quenching greatly exceeds that of chemical quenching and involves the transfer of excitation energy from $^1O_2$ to the carotenoid, resulting in ground-state oxygen and excited triplet-state carotenoid (reaction 11). The energy is dissipated through rotational and

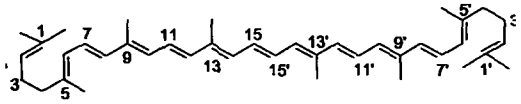
vibrational interaction between the excited carotenoid and surrounding solvent to yield the ground state carotenoid and thermal energy (reaction 12).

$^1O_2$ + Carotenoids $\rightarrow$ $^3O_2$ + ^{3} Carotenoids (reaction 11)

^{3} Carotenoids $\rightarrow$ Carotenoids + heat (reaction 12)

The quenching ability of carotenoid mainly depends on the number of conjugated double bonds and is influenced to a lesser extent by carotenoid end groups (cyclic or acyclic) or the nature of substituents in carotenoids containing cyclic groups. Lycopene (11 conjugated and two nonconjugated double bonds) is among the most efficient $^1O_2$ quenchers of the natural carotenoids (Figure 3) (42).

FIGURE 3



In the following experiments two different sources of $^1O_2$ were employed: (a) photosensitization with polymer-bound Rose bengal (Sensitox®) or with Chelex® resine-associated methylene blue (MB-Chelex®) through a type II photoreaction (Figure 2) (48); (b) thermal decomposition of the water-soluble endoperoxide $NDPO_2$ excluding the type I photoreaction (reaction 3) (20).

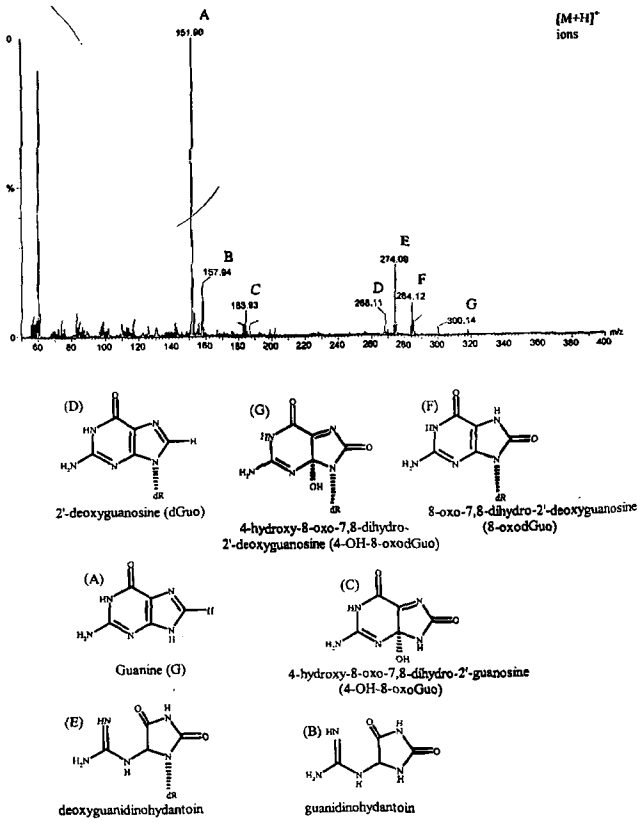
Experimental procedures

Carotenoids entrapped in human albumin: Carotenoids were dissolved in 100 µL of destilled THF/0.025% BHT. Five µL of this mixture was added to a solution of human albumin (HSA) (1 mg/mL) every 5 min for 20 min. The amount of carotenoid associated with albumin was determinated spectrophotometrically after extraction in chloroform (ε_{456} ß-carotene = 128,800 $M^{-1}cm^{-1}$ and ε_{473} lycopene = 199,526 $M^{-1}cm^{-1}$).

Detection of 8-oxodGuo and 4-OH-8-oxodGuo: The amount of 8-oxodGuo present in the solution was analyzed in a HPLC, Shimadzu (Kyoto, Japan) system, connected to a UV, set at 285 nm, and an electrochemical detectors at a potential of + 650 mV. A reversed phase column C-18 (Spherex, 250 x 4.6 mm, 5 µm) was used and the mobile phase was KH_2PO_4, 50 mM, pH 5.5 with 10% methanol and 2.5 mM EDTA (34). The 4-OH-8-oxodGuo was measured by HPLC and UV detector using a normal phase amino substituted silica gel Hypersil NH_2 column (250 x 4.6 mm, 5 µm) and a mobile phase consisting of a mixture of 25 mM ammonium formate and acetonitrile (40:60). Electrospray ionization mass spectrometry was also used to identify the oxidation products of dGuo after reaction with $^1O_2$. Samples were analyzed with a Quattro II (Micromass, Manchester, U.K.) mass spectrometer with an electrospray ion source.

A 10 µl sample of the mixture of 1mM dGuo and 1 O.D. (optical density) MB in H_2O, pH 7.0 irradiated for 30 minutes, was injected. Positive ion electrospray (LC-ESI-MS) were recorded at a capillary voltage of 3.5 kV, a cone voltage of 50 V and a source temperature of 80°C. Data were processed with MassLynx software. Positive-ion electrospray spectra exhibited $[M+H]^+$ ions. The mass spectrum obtained (Figure 4) exhibited peaks at m/z = 268.11, 284.12 and 300.14 attributed to $[M+H]^+$ of dGuo, 8-oxodGuo (49) and 4-OH-8-oxodGuo, respectively. The peak at m/z =274.09 is attributed to 2'-deoxyguanidinohydantoin (39, 50). The loss of the sugar ring produced peaks at m/z = 151.90, 157.94 and 183.93 corresponding to guanine, guanidinohydantoin, 4-OH-8-oxoGuo, respectively.

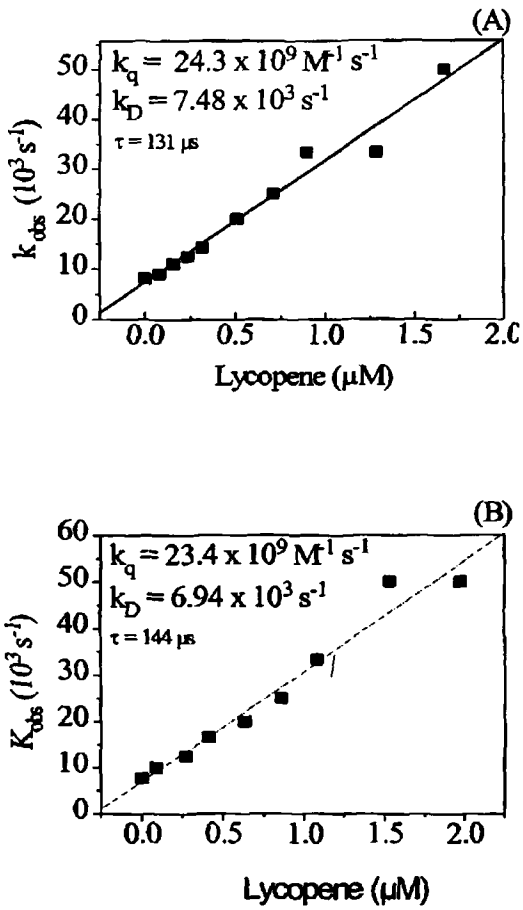
FIGURE 4



Reaction systems: System 1: Time-resolved infrared luminescence technique. Since $^1O_2$ can be generated by light excitation of a specific sensitizer in an oxygenated media, pulsed light sources have been used in order to generate a burst of such species. Then, its decay to ground state or products can be followed by any monitoring system that senses directly its

presence. By the end of the 70's, it became widely recognized that $^1O_2$ elicits phosphorescence at 1270 nm region due to its $^1\Delta g \rightarrow {}^3\Sigma_g^-$ transition (27,51) (reaction 5), with the use of fast response Germanium-based photodiodes coupled to amplifiers of appropriate bandwidth and gain, after a laser pulse excitation of a sensitizer. The most used excitation systems are Q-switched, frequency doubled or tripled Nd:YAG lasers (λ excitation = 532 and 355 nm, respectively). The signal from the diode can be fed into a box-car or transient digitizer oscilloscope. Precise determination of lifetimes ($k_D = 1/\tau$ $^1O_2$) provides an easy and accurate method to calculate quantum yields and quenching constants (kq) using Stern-Volmer plots. The kq is the slope of a plot of k_{obs} *vs* the concentration compound (K_{obs} is the observed rate constant) (Figure 5) (52). Time resolved near-infrared luminescence of $^1O_2$ was obtained by collecting the 1270 nm light emitted at right angle using a liquid nitrogen cooled germanium photodiode (EG&G Judson model J16D-M204-R05M-60), after excitation at 532 nm with a frequency doubled Q-switched Nd-YAG laser (1500 W) (Spectron Laser System). The $^1O_2$ was generated using tetraphenylporphyrin (7 ng/ml) in chloroform (53).

FIGURE 5



We investigated the quenching ability of lycopene (98% purity) (Figure 5A) and industrial tomato extracts containing 10% of lycopene in oleoresins (Figure 5B), applying the time-resolved infrared luminescence technique (52,53). Using the same concentrations of lycopene, the physical quenching rate constant (k_q) of the extracts was similar ($k_q = 24.3 \times 10^9$, $23.4 \times 10^9\ M^{-1}\ s^{-1}$, respectively), indicating that oleoresins present in the solution did not affect the quenching ability of lycopene (Figure 5). A singlet oxygen quenching constant of $31 \times 10^9\ M^{-1}\ s^{-1}$ was measured using the technique of steady state $^1O_2$ production by $NDPO_2$ and the monomol photoemission by Di Mascio et al. (41). Significant variations can arise when comparing rate constants determined by different methods. The value obtained with this technique is higher, probably due to the high lycopene quality (99.9 %).

System 2: Photosensitization with 2 mg of Sensitox® or MB-Chelex® was employed to generate $^1O_2$. One mM of dGuo was incubated in the presence of 1 mM of $FeSO_4$ in water at 37°C and irradiated during 30 minutes with a 50 W tungsten lamp placed 10 cm from the solution.

2'- Deoxyguanosine is the main target in DNA constituent that can be oxidized by $^1O_2$ through the type II photoreaction. So, this provides a good model system to evaluate the photoprotection of lycopene. Photosensitization of an aqueous solution of dGuo containing MB-Chelex® (Figures 6A, and B) or Sensitox® (Figure 7A) gave rise to the formation of 8-oxodGuo (Figure 6A, 7A and B) and 4-OH-8-oxodGuo (Figure 6B). In the presence of 300 μM lycopene associated to 0.5 mg/ml albumin, the yield of these products decreased as a function of time under irradiation due to the $^1O_2$ quenching effect of lycopene. Using MB-Chelex® and light as a source of $^1O_2$ and Calf Thymus DNA (CT-DNA) as a target, lycopene was also able to protect DNA against dGuo oxidation measured after CT-DNA hydrolysis (Figure 7B). A clear protection was observed over 10 min irradiation time.

System 3: Using the thermodissociation of 5mM $NDPO_2$ as a source of $^1O_2$, the incubation was performed in the presence of 1 mM of dGuo and different concentrations of ß-carotene or lycopene in phosphate buffer 0.1 M, pH 7.4 at 37°C (Figure 8).

In conclusion, lycopene and ß-carotene were entrapped in albumin (reactions 13 and 14) to make possible the interaction of these carotenoids with $^1O_2$ in aqueous solution. Lycopene was a better quencher of $^1O_2$ than ß-carotene in protecting dGuo from $^1O_2$ generated by $NDPO_2$, a chemical generator, or MB-Chelex®, a photosensitizer.

$^1O_2$ + Carotenoids-HSA→ $^3O_2$ + *Carotenoids-HSA (reaction 13)

*Carotenoids-HSA → Carotenoids-HSA + heat (reaction 14)

FIGURE 6

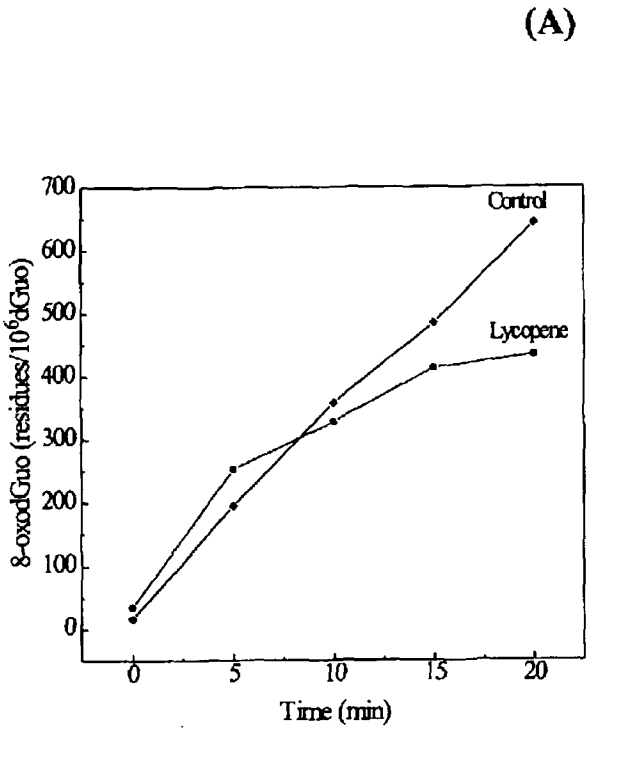


FIGURE 7

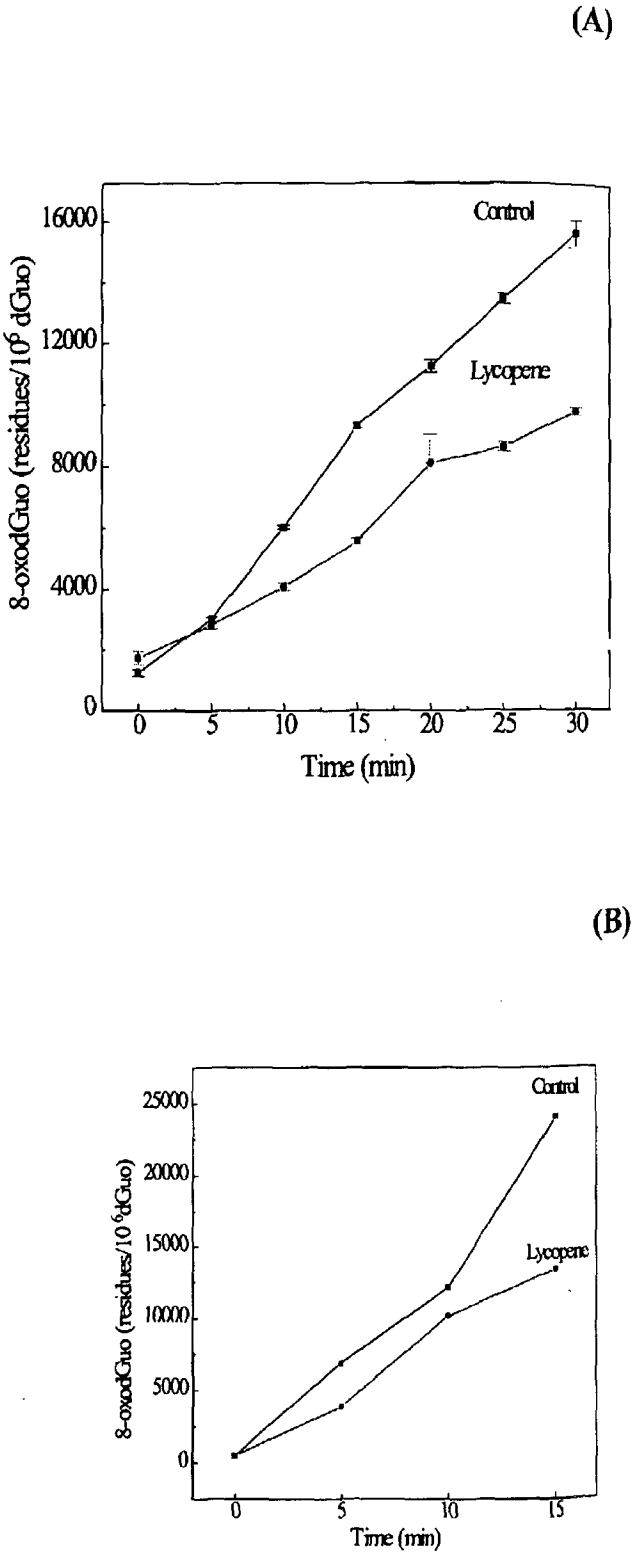
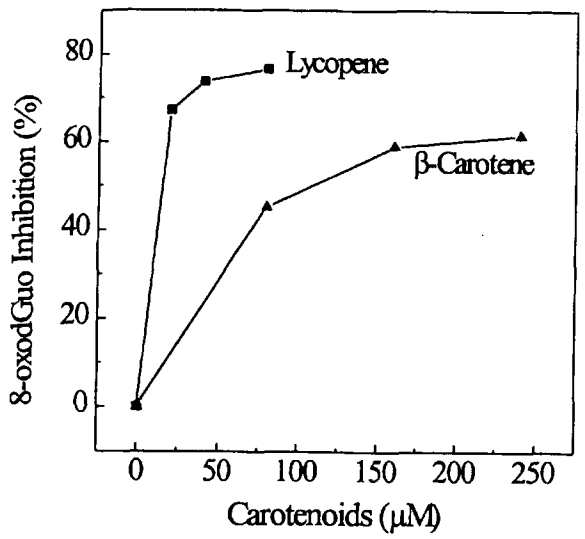


FIGURE 8



The quenching efficiency of lycopene may be of interest in protecting against $^1O_2$-induced damage of biological macromolecules like DNA, as may occur in lung oxidant injuries, skin photosensitivity, erythropoietic porphyria and toxicity of certain photosensitizers used in photochemotherapy.

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REFERENCES

1. Kasha M. Singlet oxygen electronic structure and energy transfer. In: Frimer AA, editor. Singlet O_2 Vol I. Boca Raton: CRC Press, 1985:1-14.
2. Gollnick K. Photoxygenation reactions in solution. Adv Photochem 1968; 6:1-122.
3. Foote CS. Photosensitized oxidation and singlet oxygen: consequences in biological systems. In: Pryor WA, editor. Free radicals in biology Vol 2. NY: Academic Press, 1986: 85-133.
4. Ogryzlo EA. Gaseous singlet oxygen. In: Wasserman HH, Murray RW, editors. Singlet oxygen. NY: Academic Press, 1979:35-56.
5. Murray RW, Kaplan ML. Singlet oxygen sources in ozone chemistry. Chemical oxygenations using the adducts between phosphite esters and ozone. J Am Chem Soc 1969; 91: 5358-64.
6. Khan AU. Singlet molecular oxygen from superoxide anion and sensitized fluorescence of organic molecules. Science 1970; 168: 476-7.
7. Aubry JM, Rigaudy J, Ferradini C, Pucheault J. A search for singlet oxygen in the disproportionation of superoxide anion. J Am Chem Soc 1981; 103: 4965-6.
8. Corey EJ, Mehrotra MM, Khan AU. Water induced dismutation of superoxide anion generates singlet molecular oxygen. Biochem Biophys Res Commum 1987; 145: 842-6.
9. Corey EJ, Mehrotra MM, Khan AU. Antiarthritic gold compounds effectively quench electronically excited singlet oxygen. Science 1987; 236: 68-9.
10. Fridovich I, Handler P. Xanthine oxidase. IV. Participation of iron in internal electron transport. J Biol Chem 1958; 233: 1581-5.
11. Held AM, Halko DJ, Hurst JK. Mechanisms of chlorine oxidation of hydrogen peroxide. J Am Chem Soc 1978; 100: 5732-40.
12. Moro MA, Darley-Usmar VM, Goodwin DA, Read NG, Zamora-Pinto R, Feelisch M, et al. Paradoxical fate and biological action of peroxynitrite on human platelets. Proc Natl Acad Sci USA 1994; 91: 6702-6.
13. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524-6.
14. Di Mascio P, Bechara EJH, Medeiros MHG, Briviba K, Sies H. Singlet molecular oxygen production in the reaction of peroxynitrite with hydrogen peroxide. FEBS Letters 1994; 355: 287-9.
15. Sharov VS, Driomina ES, Briviba K, Sies H. Sensitization of peroxynitrite chemiluminescence by the triplet carbonyl sensitizer coumarin-525. Effect of CO2. Photochem Photobiol 1998; 68: 797-801.
16. Moureu C, Dufraisse C, Dean PM. Sur un hydrocarbure coloré: le rubrène. C R Acad Sci 1926; 182: 1440-3.
17. Dufraisse C, Velluz L. L'union labile de l'oxygène au carbone: un peroxyde spontanément dissociable à froid. C R Acad Sci 1939; 208: 1822-4.
18. Rigaudy J, Guillaume J, Maurette D. Effet des substituants sur la photoxygénation des dérives aromatiques. Formation simultanée de photoxydes isomères en 1-4 et 9-10 à partir de diméthyl-1,4 -anthracènes. Bull Soc Chim Fr 1971; 144-52.
19. Wasserman HH, Larsen DL. Formation of 1,4-endoperoxides from the dye-sensitized photo-oxygenation of alkyl-naphthalenes. J C S Chem Comm 1972: 253-4.
20. Di Mascio P, Sies H. Quantification of singlet oxygen generated by thermolysis of 3,3'-(1,4-naphthylidene)dipropionate. Monomol and dimol photoemission and the effects of 1,4-Diazabicyclo[2.2.2]octane. J Am Chem Soc 1989; 111: 2909-14.
21. Sies H, Menck CFM. Singlet oxygen induced DNA damage. Mutation Res 1992; 275: 367-75.
22. Sies H. Damage to plasmid DNA by singlet oxygen and its protection. Mutation Res 1993; 299: 183-91.
23. Menck CFM, Di Mascio P, Agnez LF, Ribeiro DT, Costa de Oliveira R. Genetic deleterious effects of singlet oxygen. Quím Nova 1993; 16: 328-36.
24. Kasha M, Khan AU. The physics, chemistry, and biology of singlet molecular oxygen. Ann NY Acad Sci 1970; 171: 5-9.

25. Boveris A, Cadenas E, Chance B. Ultraweak chemiluminescence: a sensitive assay for oxidative radical reactions. Fed Proc 1981; 40: 195-8.
26. Cadenas E, Sies H. Low-level chemiluminescence as an indicator of singlet molecular oxygen in biological systems. Meth Enzymol 1984; 105: 221-31.
27. Khan AU, Kasha M. Direct spectroscopic observation of singlet oxygen emission at 1268 nm excited by sensitizing dyes of biological interest in liquid solution. Proc Natl Acad Sci USA 1979; 76: 6047-9.
28. Khan AU. Direct spectral evidence of the generation of singlet molecular oxygen ($^1\Delta_g$) in the reaction of potassium superoxide with water. J Am Chem Soc 1981; 103: 6516-7.
29. Bloodworth AJ, Eggelte HJ. Endoperoxides. In: Frimer AA, editor. Singlet O_2 Vol II. Boca Raton: CRC Press, 1985: 93-204.
30. Alder K, Pascher F, Schmitz A. Substituting additions I. Addition of maleic anhydride and azodicarboxylic esters to singly unsaturated hydrocarbons. Substitution processes in the allyl position. Ber Dtch Chem Ges 1943; 76: 27-8.
31. Baumstark AL. The 1,2-dioxetane ring system: preparation, thermolysis, and insertion reactions. In: Frimer AA, editor. Singlet O_2 Vol II. Boca Raton: CRC Press, 1985: 1-38.
32. Kajiwara T, Kearns DR. Direct spectroscopic evidence for deuterium solvent effect on the lifetime of singlet oxygen in water. J Am Chem Soc 1973; 95: 5886-90.
33. Bielski BHJ, Saito E. Deuterium isotope effects on the decay kinetics of perhydroxyl radical. J Phys Chem 1971; 75: 2263-6.
34. Sies H. Biochemistry of oxidative stress. Angew. Chem. (Inst. Ed. Engl.) 1986; 25: 1058-71.
35. Di Mascio P, Medeiros MHG, Bechara EJH, Catalani LH. Singlet molecular oxygen: Generation, reactivity, identification and biological effects. Ciênc C 1995; 47: 297-311.
36. Cadet J, Berger M, Douki T, Ravanat J-L. Oxidative damage to DNA: formation, measurement, and biological significance. Rev Physiol Biochem Pharmacol 1997; 131: 1-87.
37. Ravanat J-L, Cadet J. Reaction of singlet oxygen with 2'-deoxyguanosine and DNA. Isolation and characterization of the main oxidation products. J Chem Res Toxicol 1995; 8(3): 379-88.
38. Cadet J, Berger M, Douki T, Morin B, Raoul S, Ravanat J-L, Spinelli S. Effects of UV and visible radiation on DNA-final base damage. Biol Chem 1997; 378: 1275-86.
39. Goyal RN, Jain N, Garg K. Electrochemical and enzymatic oxidation of guanosine and 8-hydroxyguanosine and the effect of oxidation products in mice. Bioelectrochem Bioeng 1997; 43: 105-14.
40. Foote CS. Quenching of singlet oxygen. In: Wasserman HH, Murray RW, editors. Singlet oxygen. NY: Academic Press, 1979: 139-71.
41. Di Mascio P, Sundquist A, Devasagayam TA, Sies H. Assay of lycopene and other carotenoids as singlet oxygen quenchers. Meth Enzymol 1992; 213: 429-38.
42. Di Mascio P, Kaiser S,. Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 1989; 274: 532-8.
43. Rabek JF. $^1O_2$ oxidation of polymers and their stabilization. In: Frimer AA, editor. Singlet O_2 Vol IV. Boca Raton: CRC Press, 1985: 1-90.
44. Khan AU, Mei YH, Wilson T. A proposed function for spermine and spermidine: protection of replicating DNA against damage by singlet oxygen. Proc Natl Acad Sci USA 1992; 89: 11426-7.
45. Khan AU, Di Mascio P, Medeiros MHG. Wilson T. Spermine and spermidine protection of plasmid DNA against single-strand breaks induced by singlet oxygen. Proc Natl Acad Sci USA 1992; 89:11428-30.
46. Sies H, Stahl W. Lycopene: Antioxidant and biological effects and its bioavailability in the human. Proc Soc Exp Biol Med 1998; 218: 121-4.
47. Gerster HMA. The potential role of lycopene for human health J Am Coll Nutr 1997; 16, 109-26.
48. Greenwald RA. Handbook of methods for oxygen radical research. Boca Raton: CRC Press, 1985.
49. Ravanat J-L, Duretz A, Guiller T, Douki T, Cadet J. Isotope dilution high-performance liquid chromatography-electrospray tandem mass spectrometry assay for the measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine in biological samples. J Chromatogr B 1998; 715: 349-56.
50. Burrows CJ, Muller JG. Oxidative nucleobase modifications leading to strand scissions. Chem Rev 1998; 98: 1109-51.
51. Krasnovsky Jr AA. Photoluminescence of singlet oxygen in pigment solutions. Photochem Photobiol 1979; 29: 29-36.
52. Wilkinson F, Helman WP, Ross AB. Quantum yields for the photosensitized formation of the lowest electronically excited singlet state of molecular oxygen in solution. J Phys Chem Ref Data 1993; 22: 113-262.
53. Di Mascio P, Medeiros MHG, Sies H, Bertolotti S, Braslavsky SE, Veloso DP, et al. Quenching of singlet molecular oxygen by natural furan diterpenes. Photochem Photobiol 1997; 38: 169-73.

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Bioavailability of carotenoids

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SUMMARY. Our current knowledge about the bioavailability of provitamin A carotenoids in foods is insufficient, fragmentary and difficult to interpret. Past methods of estimating the vitamin A value of food carotenoids suffer both from uncertainty about the meaning of bioavailability and from the inadequacy of the indicators used in its determination. Reported conversion ratios of ß-carotene to vitamin A in humans *in vivo*, depending on conditions, range from 2:1 to 26:1 (µg/µg). Thus, the ratio of 6:1, devised by the World Health Organization, must be considered as a rough average estimate that is not applicable to all diets. Strategies to increase the dietary intake of carotenoid-containing foods should include measures to enhance carotenoid bioavailability.
Key-words: Carotenoids, bioavailability, vitamin A value.

RESUMEN. Biodisponibilidad de carotenoides. Nuestros conocimientos actuales sobre la biodisponibilidad de los carotenoides en alimentos son insuficientes, fragmentados y difíciles de interpretar. Métodos pasados para calcular el valor de vitamina A de los carotenoides alimenticios padece tanto de la incertidumbre sobre el significado del término biodisponibilidad como de la inadecuacidad de los indicadores usados en su determinación. Las razones reportadas para la conversión del ß-caroteno en vitamina A en humanos *in vivo*, dependiendo de las condiciones, varían de 2:1 a 26:1 (µg/µg). Así, la razón de 6:1, establecida por la Organización Mundial de la Salud, debe ser considerada como un estimativo promedio aproximado que no se aplica para todas las dietas. Las estrategias para aumentar el consumo de los alimentos que contienen carotenoides deben incluir medidas para realzar la biodisponibilidad de los carotenoides.
Palabras clave: Carotenoides, biodisponibilidad, valor de vitamina A.

INTRODUCTION

The best utilized ingested form of vitamin A is as an ester of retinol in an oily solution. The ester passes intact through the mouth and stomach, is hydrolyzed to retinol in the upper intestine, and is absorbed as a micelle into the intestinal mucosa (1). Retinol is subsequently esterified with long-chain fatty acids, and particularly with palmitic acid, is incorporated into chylomicra, and is secreted into the lymph. Upon reaching the systemic circulation, the triglyceride of the chylomicron is hydrolyzed in large part to glycerol and fatty acids by lipoprotein lipase, and the resultant chylomicron remnant is taken up by the liver as well as, to a minor degree, by other tissues. In the liver, retinol is reesterified and stored for subsequent use, primarily in stellate cells but also in hepatocytes. Under normal dietary conditions, preformed vitamin A is absorbed very well and is stored efficiently (1).

Preformed vitamin A is found primarily in foods of animal origin (1). Vitamin A can also be formed, however, from a specific set of plant pigments known as carotenoids (1,2). Over 600 carotenoids exist in nature, of which approximately 50 serve as precursors of vitamin A. Because the vast majority of humans obtain their vitamin A from these carotenoid precursors, carotenoid bioavailability is an important public health consideration in most of the world. Carotenoids are less bioavailable than preformed vitamin A in food. First of all, they are bound rather tightly within the matrix of the fruit or vegetable. Thus, they must be released from the matrix by digestive processes. Secondly, their requirements for absorption into intestinal cells are more demanding than for vitamin A. Thirdly, they must be enzymatically cleaved into vitamin A within intestinal cells or within other cells of the body. And, fourthly, they must be stored either as vitamin A or as carotene itself within various tissues. The bioavailability of carotenoids in foods has varied over ten-fold from one study to another (2).

At the outset, it is useful to define the concept of bioavailability (3). Absolute bioavailability is defined as the proportion of an ingested dose that is absorbed in a biologically useable form. Absolutely bioavailability can be determined fairly readily for nonmetabolizeable compounds, such as metals, but is much more difficult for metabolizeable organic substances like carotenoids. Thus, absolute bioavailability for carotenoids has never been determined. Relative bioavailability is the percentage of the substance that is absorbed in a biologically utilizeable form relative to a reference substance treated in the same manner. Obviously, comparative studies are much easier to conduct. The reference substance has usually been either ß-carotene in oil or retinyl ester in a readily bioavailable form. The most commonly used indicator of bioavailability has been changes in plasma retinol in vitamin

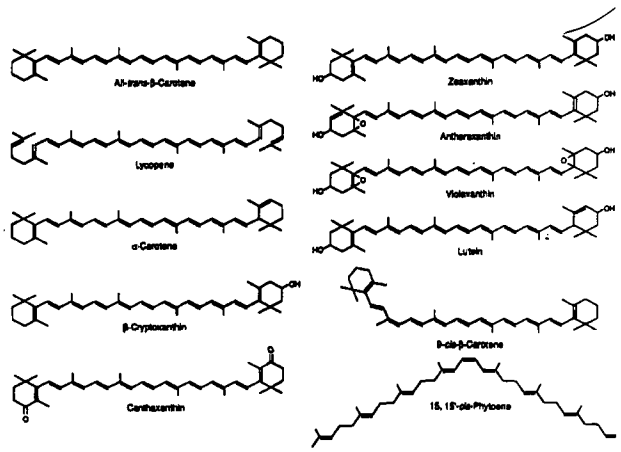
A-depleted subjects in response to increased intake of a known amount of a carotenoid.

Two facets of carotenoid bioavailability exist: Firstly, its absorption into intestinal cells and secondly, its conversion into vitamin A. In practice, these two facets are very difficult to separate. Thus, the absolute bioavailability of a single known dose of a provitamin A carotenoid is defined as the percentage amount of vitamin A-active substances formed from it. Similarly, the relative bioavailability concerns the response of an appropriate indicator to a carotenoid, usually in food, relative to a reference substance.

Structures

The structures of some common carotenoids are shown in Figure 1 (4). The parent compound, all-*trans* ß-carotene, is the most common provitamin A carotenoid found in foods. Its cis isomers, including 9-*cis* ß-carotene, is less active biologically than the all-*trans* form. Furthermore, other carotenoids that contain a single ß-ionone ring, like ß-cryptoxanthin and α-carotene, are approximately 50% as active in biological testing. The other carotenoids shown, which do not serve as precursors of vitamin A, nonetheless are common constituents of foods. Indeed, lutein and lycopene, at least in the United States, are present in plasma in higher concentrations than is ß-carotene. These other carotenoids, which may have protective aspects relative to health, are not precursors of vitamin A (2).

FIGURE 1
Some common naturally occurring carotenoids (4)



Intakes

The intakes of total vitamin A, including preformed vitamin A and provitamin A carotenoids, vary tremendously in different areas of the world (5). In general, populations in most industrialized areas of the world have adequate intakes of approximately 1 mg of preformed vitamin A and 2 mg of ß-carotene or its equivalent. In less industrialized countries, the total amount of vitamin A ingested is less, and the percentage derived from provitamin A carotenoids is much higher. Vitamin A deficiency, as indicated by clinical manifestations, has most commonly been found in Africa and Asia, although, at one time, certain areas of Central America and South America were also affected. Although clinical vitamin A deficiency tends to be decreasing in most countries of the world, the World Health Organization nonetheless estimates that the health of more than 250,000,000 preschool children is compromised by vitamin A inadequacy (5,6).

Cleavage

The bioavailability of carotenoids initially depends on the way in which they are converted into vitamin A or other products. Carotenoids can be cleaved centrally to yield, in the case of ß-carotene, two molecules of retinal. ß-Carotene might also be cleaved eccentrically to yield ß-apo-carotenals, which then can be oxidatively converted to one molecule of retinal. Finally, carotenoids can be oxidatively destroyed by free radicals and by enzymes such as lipoxygenase. In healthy individuals with an adequate vitamin A status, the major pathway for the formation of vitamin A from provitamin A carotenoids is central cleavage, which is carried out by an enzyme, ß-carotene-15,15'-dioxygenase, that is present in the cytosol of the intestine and of many other organs (7,8). The properties of this enzyme are given in Table 1. Because of its instability, the enzyme has not yet been highly purified nor cloned.

TABLE 1
Properties of ß-carotene 15,15'-dioxygenase
(EC 1.13.11.21)

Cleaves many provitamin A carotenoids
Cytosolic
Requires molecular oxygen
Gives two moles of retinal per mole of ß-carotene cleaved
K_M = 1-10 μM
pH_o = 7.5-8.5
Requires a detergent
Needs free sulfhydryl groups
Probably contains iron or copper
Present in both neonatal and adult intestinal cells, liver and many other tissues
Associated with CRBP-II concentration

Equivalencies

As a result of growth responses in vitamin A deficient rats, an international unit (IU) was defined as 0.3 μg of all-*trans* retinol in oil or 0.6 μg of all-*trans* ß-carotene in oil (9). Thus, in *in vivo* studies, 1 μg of all-*trans* retinol was equivalent to 2 μg of all-*trans* ß-carotene. A two-fold difference therefore exists between the cleavage ratio, where 1 μg of ß-carotene yields an equivalent amount of all-*trans* retinol, and the ratio *in vivo*, where twice as much ß-carotene is required. This difference can be attributed to the poorer intestinal absorption

of ß-carotene relative to vitamin A and the conversion of a discrete portion of the retinal formed from ß-carotene to retinoic acid, which is not usually measured and is rapidly metabolized. These findings in animals were confirmed in humans in a vitamin A depletion-repletion study with male volunteers (10).

As already indicated, ß-carotene in foods is less biologically active than ß-carotene in oil. Thus, in 1967 the World Health Organization (WHO) suggested that a ratio of 6 µg all-*trans* ß-carotene in food is equivalent to 1 µg all-*trans* retinol in food (11). The retinol equivalent (RE) was thereby established, which could be expressed in any mass units, i.e., ng, µg, mg, etc. Generally, the µg RE is most employed. The WHO committee based their equivalency ratio on two assumptions; namely, (a) that the mass ratio of the maximum conversion of ß-carotene in oil, its most bioavailable form, to vitamin A *in vivo* was 2:1 and (b) that the bioavailability of ß-carotene from foods on the average was one-third that of ß-carotene in oil. Thus, 2 x 3 = 6 µg ß-carotene per µg retinol. Other common carotenoids, like ß-cryptoxanthin and α-carotene, were considered to be half as active as ß-carotene. *Cis*-isomers were later considered as well to be half as active as their all-*trans* counterparts.

The WHO expert committee based their 3:1 ratio primarily on balance studies in which the fecal content of carotenoids was subtracted from the amount ingested to give the amount absorbed. This method tends to overestimate the amount of the carotenoid absorbed because of the poorer extraction efficiency of carotenoids from foods than from oil and of probable destruction of carotenoids in the GI tract. Nonetheless, the expert committee did make a correction for these confounding factors. In support of their conclusions, mass conversion ratios of 5.8:1 and 6.4:1 for ß-carotene in papaya and in amaranth, respectively, can be calculated from the results of a well-designed study with preschool children in India (12).

On the basis of many similar studies conducted in India, a conversion ratio of 4:1 for the food based ß-carotene/vitamin A conversion was accepted as part of the Indian dietary allowances (13). The approach used was similar to that employed by the WHO, but the average bioavailability of ß-carotene in ingested vegetables and fruits, relative to ß-carotene in oil, was considered to be 50%, not 33.3%.

In 1995, lactating Indonesian women were fed either stir-fried vegetables containing 3.5 mg of ß-carotene and 7.8 g of fat, an enriched wafer containing the same amount of ß-carotene in a highly bioavailable form together with fat, or a control wafer containing no ß-carotene (14). In those receiving the enriched wafer, serum retinol, breast milk retinol and serum ß-carotene increased significantly, whereas in those given either stir-fried vegetables or the control wafer, no positive response was noted. Thus, the public health strategy of enhancing the intake of carotenoid containing fruits and vegetables as a means of improving vitamin A status was questioned. Later, in a similar study carried out with Indonesian children, ratios of 26 µg of ß-carotene in vegetables or 12 µg of ß-carotene in fruits were calculated to be equivalent to 1 µg of retinol on the basis of changes in serum retinol (15). These latter ratios are probably too high, inasmuch as no correction was made for absorbed but not immediately converted provitamin A carotenoids. Indeed, in some cases, up to 60% of absorbed ß-carotene is not immediately converted into vitamin A (16).

Another way of looking at this issue is to compare the intake of carotenoids to the basal vitamin A requirement of growing children. The basal vitamin A requirement, as defined by FAO/WHO, is the amount of vitamin A that is needed for growth and the maintenance of various physiological responses but without leading to significant vitamin A storage (17). In the case of 3- to 5-year old children, the basal requirement is approximately 216 µg retinol equivalents. In feeding studies involving amaranth and papaya in India, in which ratios of 5.8 and 6.4 µg ß-carotene/µg retinol were obtained, the basal vitamin A requirement is fully met (12). On the other hand, if ratios of 26:1 for vegetables or 12:1 for fruits are used (15), only 1/4 or 1/2 of the basal vitamin A requirement would be met by the provided diets. These children, however, grew normally and plasma retinol values rose under this feeding regimen (12).

A variety of new methods are now being used to gain greater insight into carotenoid bioavailability. These include area-under-the-curve studies with isotopically labelled ß-carotene, vegetables and vitamin A (18); sophisticated kinetic models involving a variety of compartments after the ingestion of labeled ß-carotene (19); careful area-under-the-curve measurements of carotenoids and vitamin A in the triglyceride-rich fraction of plasma after a single dose of ß-carotene in oil or foods (16); the measurement of total body stores of vitamin A employing isotope dilution techniques before and after increased ingestion of carotenoid-rich foods (20); and steady-state isotope-dilution procedures with labeled carotenoids and vitamin A (21).

Influences

Several factors influence the conversion of ß-carotene and other provitamin A carotenoids into vitamin A. These factors might be considered in two groups; namely: (a) those that influence the activity of the major cleavage enzyme, ß-carotene-15,15'-dioxygenase, and (b) those that influence in large part the absorption of provitamin A carotenoids from the GI tract.

ß-Carotene-dioxygenase shows increased activity in the presence of high intakes of fat and particularly of polyunsaturated fat, high intakes of protein and high intakes of ß-carotene (22,23). The activity is increased as well when the vitamin A status is poor. The enzyme activity is decreased by factors opposite to those just cited.

Exogenous factors that influence carotenoid bioavailability (2,24) are summarized in Table 2. The absorption efficiency of all-*trans* ß-carotene in oil is usually 20-60%. The absorption

efficiency declines rapidly, however, as the amount ingested increases. In general, hydrocarbon carotenoids are fairly well absorbed as are xanthophylls and apo-carotenoids. The all-*trans* isomers clearly are better absorbed than the *cis* isomers and diepoxides and allene carotenoids tend to be poorly absorbed. When present together in the GI tract, carotenoids tend to compete with each other for absorption (25,26). Thus, ß-carotene seems to inhibit canthaxanthin and lutein absorption, at least when present together in large amounts, but may enhance lycopene absorption. Oxo-carotenoids seem to affect ß-carotene absorption to a lesser degree.

TABLE 2
Factors affecting carotenoid bioavailability

* Amount ingested
* Carotenoid structure
* Competition among carotenoids
* Physical state
* Dietary fat, fiber, oxidants and antioxidants
* Food preparation and particle size
* Stomach acidity and the composition and flow of bile
* Lipid malabsorption
* Infections
* Genetic factors

The physical state of the carotenoid is a key issue, in that carotenoids in oil or in specially prepared beadlets are very well absorbed, whereas those in fruits and vegetables are absorbed to a lesser degree. The presence of dietary fat and antioxidants in the GI tract enhances ß-carotene stability and absorption, whereas fiber and oxidants have the opposite effects. Cooked, pureed carrots and spinach seem to be absorbed approximately 3-fold better than the raw, intact vegetables (27). In some instances, this ratio can be even higher. A reduced rate of bile flow, a decreased amount of cholic acid analogs and stomach acidity will also influence carotenoid absorption.

Any of a series of lipid malabsorption syndromes will of course markedly reduce the absorption of carotenoids (1,2). Infections, either systemic or from intestinal parasites, will also adversely affect carotenoid uptake. Finally, genetic factors clearly influence all aspects of carotenoid and vitamin A metabolism. These factors, however, have not been quantitated in any effective way.

FINAL REMARKS

The bioavailability of food carotenoids as sources of vitamin A is affected by a host of endogenous and exogenous factors. Clearly, no single conversion factor suitable for all foods and conditions can be defined. Ways of preparing and storing carotenoid containing foods to enhance their bioavailability should be devised and taught to populations at risk. Quite possibly, different conversion ratios might be employed for dark green leafy vegetables, fruits, carotenoids in oily solutions such as red palm oil, and so on, in order to better estimate the actual bioavailability of carotenoids in a diet. Clearly, the carotenoid content of ingested foods in a given culture might well differ markedly from values found in food composition tables (28). Nonetheless, with the application of new experimental approaches, better information concerning this important nutritional issue should be forthcoming in the near future.

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REFERENCES

1. Ross AC. Vitamin A and retinoids. In: Shils ME, Olson JA, Shike M, Ross, AC, editors. Modern nutrition in health and disease, 9th ed. Baltimore: Williams & Wilkins, 1999: 305-27.
2. Olson JA. Carotenoids. In: Shils ME, Olson JA, Shike M, Ross AC, editors. Modern nutrition in health and disease, 9th ed. Baltimore: Williams & Wilkins, 1999: 525-41.
3. Parker RS. Bioavailability of carotenoids. Eur J Clin Nutr 1997; 51: 86-90.
4. Olson JA, Krinsky NI. The colorful, fascinating world of the carotenoids: important physiologic modulators. FASEBJ 1995; 9: 1547-50.
5. Underwood BA. Vitamin A in human health: public health considerations. In: Sporn MB, Roberts AB, Goodman DS, editors. The retinoids: biology, chemistry, and medicine. New York: Raven Press, 1994: 211-27.
6. Underwood BA, Arthur P. The contribution of vitamin A in public health. FASEB J 1996; 10: 1040-8.
7. Devery J, Milborrow BV. ß-carotene-15,15'-dioxygenase (EC 1.13.11.21): isolation, reaction mechanism and an improved assay procedure. Brit J Nutr 1994; 72: 397-414.
8. Nagao A, During A, Hoshino C, et al. Stoichiometric conversion of all-*trans* ß-carotene to retinal by pig intestinal extract. Arch Biochem Biophys 1996; 328: 57-63.
9. Moore T. Vitamin A. Amsterdam: Elsevier, 1957.
10. Sauberlich HE, Hodges RE, Wallace DI, et al. Vitamin A metabolism and requirements in the human studied with use of labeled retinol. Vitam Horm (USA) 1974; 32: 251-75.
11. Food and Agriculture Organization/World Health Organization. Requirements of vitamin A, thiamine, riboflavin, and niacin. Report of a joint FAO/WHO Expert Group. FAO Nutr Meetings Rep Ser No. 41. Rome: FAO Press, 1967: 15-27.
12. Devadas P, Saroja S, Murthy NK. Availability of ß-carotene from papaya fruit and amaranth in preschool children. Indian J Nutr Dietet 1980; 17: 41-4.
13. Gopalan C, Narasinga Rao BS. Dietary allowances for Indians. ICMR Sp Rep Ser No 60. New Delhi: ICMR Press 1991: 46-53.

14. De Pee S, West CE, Muhilal, Karyadi D, Hautvast JGAJ. Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. Lancet 1995; 346: 75-81.
15. de Pee S, West CE, Permaesih D, et al. Orange fruit is more effective than are dark-green leafy vegetables in increasing serum concentrations of retinol and ß-carotene in school children in Indonesia. Am J Clin Nutr 1998; 68: 1058-67.
16. van Vliet T, Schreurs WHP, van den Berg H. Intestinal ß-carotene absorption and cleavage in men: response of ß-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of ß-carotene. Am J Clin Nutr 1995; 62:110-6.
17. Food and Agriculture Organization/World Health Organization. Requirements of vitamin A, iron, folate, and vitamin B12. Report of a joint FAO/WHO expert consultation. FAO Food & Nutr Ser No 23. Rome: FAO Press, 1988: 16-32.
18. Tang G, Grusak MA, Qin J, et al. Vitamin A activity of spinach determined by isotope reference method. Abstracts, 19th IVACG meeting, 1999: 44.
19. Novotny JA, Dueker SR, Zech L, et al. Compartmental analysis of the dynamics of ß-carotene metabolism in an adult volunteer. J Lipid Res 1995; 36: 1825-38.
20. Ribaya-Mercado JD, Mazariegos M, Tang G, et al. Assessment of total body stores of vitamin A in Guatemalan elderly by the deuterated-retinol-dilution method. Am J Clin Nutr 1999; 69: 278-84.
21. van Lieshout M, West CE, Lugtenburg J, et al. Quantification of bioavailability of ß-carotene and its bioconversion to retinol using [$^{13}C_{10}$]-ß-carotene and [$^{13}C_{10}$]-retinyl palmitate. Abstracts, 19th IVACG meeting 1999: 42.
22. van Vliet T, van Vlissingen MF, van Schaik F, van den Berg H. ß-Carotene absorption and cleavage in rats is affected by the vitamin A concentration of the diet. J Nutr 1996; 126: 499-508.
23. During A, Nagao A, Terao J. ß-Carotene-15,15'-dioxygenase activity and cellular retinol-binding protein type II level are enhanced by dietary unsaturated triacylglycerols in rat intestine. J Nutr 1998; 128: 1614-9.
24. de Pee S, West CE. Dietary cartenoids and their role in combating vitamin A deficiency: a review of the literature. Eur J Clin Nutr 1996; 50: S38-S53.
25. Kostic D, White WS, Olson JA. Intestinal absorption, serum clearance, and interactions between lutein and ß-carotene when administered to human adults in separate or combined oral doses. Am J Clin Nutr 1995; 62: 604-10.
26. Paetau I, Chen H, Goh NM-Y, White WS. Interactions in the postprandial appearance of ß-carotene and canthaxanthin in plasma triacylglycerol-rich lipoproteins in humans. Am J Clin Nutr 1997; 66: 1133-43.
27. Rock CL, Lovalvo JL, Emenhiser C, et al. Bioavailability of ß-carotene is lower in raw than in processed carrots and spinach in women. J Nutr 1998; 128: 913-6.
28. Rodriguez-Amaya DB, Amaya-Farfan J. Current status of analytical methods for measuring provitamin A. Arch Latinoam Nutr 1992; 42: 180-91.

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The role of provitamin A carotenoids in the prevention and control of vitamin A deficiency

Penelope Nestel, Paula Trumbo

SUMMARY. That ß-carotene is the main source of vitamin A in fruits and vegetables has been known for many years. Many studies have been conducted to assess bioconversion of ß-carotene to vitamin A in animals. More recently, bioconversion studies using stable-isotopically labeled ß-carotene have been used to assess bioconversion in humans. The efficiency of the bioconversion of ß-carotene to vitamin A has been accepted to be six but this value may vary depending on vitamin A status and the amount of ß-carotene consumed. This paper reviews the human studies on purified ß-carotene supplements and/or consumption of fruits and vegetables conducted to ascertain whether ß-carotene can alter the vitamin A status of deficient populations. The conclusion is that data are lacking from well-designed studies to show that, with the possible exception of red palm oil, ß-carotene-rich foods are as effective as vitamin A supplements for eliminating vitamin A deficiency. Nevertheless, the data do show that ß-carotene-rich foods may be important for preventing vitamin A deficiency.
Key words: Provitamins A, vitamin A deficiency.

RESUMEN. El papel de los carotenoides provitamina A en la prevención y control de la deficiencia de vitamina A. El hecho de que el ß-caroteno es la fuente principal de la vitamina A en frutas y vegetales ha sido conocido por muchos años. Varios estudios han sido conducidos para evaluar la bioconversión del ß-caroteno en vitamina A en animales. Más recientemente, estudios de bioconversión con ß-caroteno marcado con isótopos estables han sido utilizados para evaluar la bioconversión en humanos. La eficiencia de la conversión de ß-caroteno en vitamina A aceptada es seis, sin embargo este valor puede variar dependiendo del status en vitamina A. Este articulo discute los estudios en humanos con suplementos de ß-caroteno puro o consumo de frutas y verduras, efectuados para averiguar si el ß-caroteno puede alterar el estado de vitamina A de poblaciones deficientes. La conclusión es que faltan datos de estudios bien diseñados para mostrar que, con la posible excepción del aceite de palma roja, los alimentos ricos en ß-caroteno son tan efectivos como los suplementos de vitamina A en la eliminación de la deficiencia de vitamina A. Sin embargo, los datos muestran que alimentos ricos en carotenoides pueden ser importantes para la prevención de la deficiencia de vitamina A.
Palabras clave: Provitamina A, deficiencia de vitamina A.

INTRODUCTION

The only proven physiological function of carotenoids in humans is as provitamin A (1). ß-carotene is the primary provitamin A in fruits and vegetables and is found naturally as the all-*trans* isomer. *Cis*-isomers, which exist both naturally and are formed during food preparation, have lower biological activity (2).

The average total vitamin A intake by region of the world, the percentage from provitamin A carotenoids and the prevalence of vitamin A deficiency is shown in Table 1. Provitamin A carotenoids account for between 60 and 90% of vitamin A intake with dependency on them as a source of vitamin A being particularly high in South East Asia, Africa and the Western Pacific (3). Although the prevalence of vitamin A deficiency is high throughout the developing world, its association with provitamin A carotenoid intake is complex due to the various factors that affect the bioavailability and bioconversion of carotenoids, which depend on both the food matrix and host-related factors (4).

TABLE 1
Vitamin A intake, percent from carotenoids and vitamin A deficiency by region (3)

Region	Total vitamin A (µg RE/d)	% Provitamin A carotenoids	Prevalence of vitamin A deficiency (%)
Africa	776	84	49
Americas	814	64	20
South East Asia	431	88	69
Europe	738	63	0
Eastern Mediterranean	936	63	22
Western Pacific	997	78	27

Studies have been conducted to assess the bioconversion of ß-carotene to vitamin A in animals (5). More recently, studies using stable-isotopically labeled ß-carotene have been carried out to assess bioconversion in humans. The efficiency of the bioconversion of ß-carotene to provitamin A has been accepted to be six (6) although this value may vary depending on vitamin A status, which data (7-9) suggests may be:

- more efficient when status is lower
- less efficient the more ß-carotene consumed.

Several human studies involving ß-carotene supplementation and/or consumption of fruits and vegetables have been conducted to find out whether ß-carotene can alter vitamin A status, particularly in the vitamin A deficient. Most of these studies have been conducted among children or pregnant/lactating women in developing countries.

Intervention studies

The information presented here summarizes intervention studies on children and women that were comprehensively reviewed by de Pee and West (4), and include more recent articles or abstracts. Thirty-two studies have been conducted that are divided into five groups namely, studies among children that included (a) no effective control groups, (b) a positive control group, (c) a negative control group, and (d) both a negative and positive control group; and studies among pregnant and lactating women.

Ideally, both negative and positive control groups should be included in intervention studies as the results will be more reliable, but minimally a negative control group should be included. A negative control group shows whether vitamin A status would change without the intervention due to the Hawthorn effect or seasonal variation in dietary practices. The positive control, in which the subjects receive the same amount of purified ß-carotene or vitamin A, will show the maximum effect of the intervention.

Intervention studies among children

The intervention studies that did not include adequate control groups (10-18) are listed in Table 2. The first column lists the study and site where it was conducted; the second column shows the age group studied; the third column shows baseline retinol level, where < 20 µg/dL is the cut off to define more than marginal deficiency; the fourth column states the intervention; the fifth column shows the duration of the study; and the final column shows the effect defined as "+" or "0".

TABLE 2
Studies with inadequate control groups

Study/Source/Site	Age	Retinol µg/dL	Intervention	Time	Effect
1. Pereira & Begun (10), India	2-5 y	22	GLV (1.5-2.25 MG ßC) No control	3 m	+
2.Lala & Reddy (11), India	2-6 y	na	Amaranth (1.2 mg ßC) -ve control,	15 d	+
3. Devadas & Murthy (12), India	3-5 y	21	Vegetables (1.2 mg ßC) then purified ßC (1.2 mg) No control	3 m	+
4. Devadas et al. (13), India	4-5 y	12 - 14	GLV (1.2 mg) or purified ßC (1.2 mg) -ve control,	2.5 m	+
5. Jayarajan et al. (14), India	2-6 y	20-21	Spinach (1.2 mg ßC) ± groundnut oil (5 or 10 g) No control	4 w	+
6. Charoenkiatkul et al. (15), Thailand	> 6 m	na	Ivy gourd (1.1 mg ßC) or VA (450 RE) No control	2 w	+
7. Mariath et al (16), Brazil	3-12 y	xeropthalmic	Buriti sweet (0.8 mg ßC) No control	20 d	+
8. Hussein & El-Tohamy (17), Egypt	11-13 y	34.2	Carrots (4.75 mg ßC), carrot juice (3.35 mg ßC), spinach (12.7 mg ßC) No control	2 w	0
9.Nasoetion et al. (18), Indonesia	10-13 y	23-25 37-38	Carrot soup or carrot juice (1.8 mg ßC) -ve control,	3 m	0

GLV-green leafy vegetables; ßC-ß-carotene; VA-vitamin A; na-not available; -ve control- diet without intervention

Seven of the nine studies reported an improvement in vitamin A status, but this should be interpreted with caution due to the design weaknesses:

- Study 5 did not have a control group for the effect of vegetables, but it did have one for the effect of fat intakes. The results showed a greater impact on vitamin A status

when 5 or 10 g of oil was included with the spinach, although there was no difference between these two levels, suggesting that as little as 5 g oil can improve ß-carotene absorption;

- Only three of the studies (studies 2, 5, and 6) gave adequate information on fat intakes as both the fat content of the intervention and the content of the daily diet. Five of the studies did not provide information on the fat content of the intervention (studies 1, 3, 4, and 8) and two on the fat content in the daily diet (studies 7 and 9);
- There were large differences in energy and fat intake between groups in two studies (studies 2 and 4);
- In study 7, the sweets were given as take-home supplements and no data were provided on compliance;
- Study 6 subjects were their own control who had been receiving routine vitamin supplements until the study; thus, the baseline retinol levels were variable;
- In study 3, the effects of the vegetable could not be separated from that of the ß-carotene because baseline vitamin A levels were not measured;
- In studies 2 and 4, the sample was not randomized. Moreover, in study 2 the sample size differed between the groups, while in study 4 the number in each group was only 4-5 individuals.

Of the two studies showing no effect, one (study 8) included children whose baseline retinol was on average 34 µg/dL; thus, as a group they were not vitamin A deficient. The other study (study 9) divided the sample into those having retinol levels below and above 30 µg/dl but no improvement was seen in either group.

The studies that included only a positive control (19-23) are listed in Table 3. Four of the five studies showed an improvement in vitamin A status. The positive controls included fortified salt (study 10), mega dose vitamin A (study 11) and vitamin A (studies 12-14). Besides the design limitation,

- Study 10 was not randomized, had different sample sizes among the groups, and had a large drop out rate (25%);
- The sample size in study 11 was very small.(n=4-5/group) and no information was provided on the daily diet.

TABLE 3
Studies with a positive control group

Study/Source/Site	Age	Retinol µg/dL	Intervention	Time	Effect
10. Muhilal & Karyadi (19), Indonesia	3-5 y	19-20	DGLV (1.9 mg ßC) or VA (0.3 mg)	75 d	0
11. Hussein & El-Tohamy (20), Egypt	6-13 y	17	Spinach (3.7 mg ßC) + Oil (10 g) or carrots (2.4 mg ßC) or VA (200 mg)	40 d/ 21 serv	+
12. Carlier et al. (21), Senegal	2-15 y	abn CIC	Purified ßC, VA	7 w	+
13. Rukmini (22) India	7-9 y	21-23	Red palm oil or VA (? 0.7 mg)	60 d	+
14. Manorama et al. (23)	7-9 y	24	Red palm oil (2.4 mg ßC) or VA (0.6 mg)	60 d	+

DGLV-dark green leafy vegetables; ßC-ßcarotene; VA-vitamin A; abn CIC-abnormal conjunctival impression cytology

The studies that included only a negative control (24-29) are listed in Table 4. Five of the six studies showed an improvement in vitamin A status (studies 15, 16, 18, 19, 20) and, in one study, the intervention prevented vitamin A deficiency (study 17). Yin et al's (26), Solon et al's (27), Takyi and Owusu's (28) and Persson et al's (29) results are available only in abstracts.

- Study 16 did not provide detail of the fat content of the intervention;
- Study 16 did not collect baseline data on status; thus, the difference could have existed at the outset;
- Study 17 showed serum retinol levels were maintained with the intervention, unlike in the control group, i.e., the intervention prevented vitamin A deficiency. Moreover, the study included children who were not vitamin A deficient at the baseline, which may explain why there was no improvement in vitamin A status per se;
- Study 20 did not provide detail of the fat content of the intervention. The study included children who were marginally subclinically deficient at baseline. Retinol levels increased significantly only among the group that received dark green leafy vegetables, who also had the lowest baseline retinol levels. The rise in serum ß-carotene was significant in both intervention groups and the control group.

The six studies that included both a negative and positive control group (30-35), which provide the most reliable results, are listed in Table 5. In these studies, the positive control included, vitamin A (studies 22,23,24 and 26) or purified ß-carotene (studies 21 and 25). The caveats in these studies include:

- Studies 21 and 24 had small sample sizes;

- Study 24 also had a large drop out rate (30%);
- Study 24 did not provide detail on the fat content of the intervention although the diet had 5-7 g fat, which would have helped absorption.
- The one study showing no effect (study 25) included children who were not vitamin A deficient.

TABLE 4
Studies with a negative control group

Study/Source/Site	Age	Retinol µg/dL	Intervention	Time	Effect
15. Jalal (24), Indonesia	2-7 y	17	ßC-rich foods (5.1 mg ßC), fat (25 g), deworming	24 d	+
16. Wadha et al. (25), India	7-12 y	not done	Carrots/papaya/coriander (2.3-3.3 mg ßC)	1 m	+
17. Yin et al. (26), China	5.5-6.5 y	33	DGLV (200 g/d)	10 w	+
18. Solon et al. (27), Philippines	Sch c'dren	VAD	Yellow fruits/veg (5 mg ßC)	5 d/w/ 12 w	+
19. Takyi and Owusu (28), Ghana	2-6 y	17-22	DGLV (2.4 mg ßC) or purified ßC (2.4 mg)	12 w	+
20.Persson et al. (29), Bangladesh	8-12 y	24-27	DGLV (3.5 mg ßC) or sweet pumpkin (1.6 mg ßC)	6 d/w/ 6w	+ 0

DGL-dark green leafy vegetables; ßC-ß-carotene; VAD-vitamin A deficiency.

TABLE 5
Studies with both a negative and positive control group

Study/Source/Site	Age	Retinol µg/dL	Intervention	Time	Effect
21. Roels et al. (30), Rwanda	9-16 y	32-36	Carrots (19 mg ßC) ± fat (18 g) or purified ßC (28 mg) + fat	31 d	+
22. Roels et al. (31), Indonesia	3-13 y	Mgnal	Palm oil (7.8 mg) or VA (0.6 mg)	22 d	+
23. Lian et al. (32), Indonesia	1-5 y	13-18	Palm oil (1.8 mg ßC) or VA	11-14 m	+
24. Devadas et al. (33), India	3-5 y	13-14	Papaya or amaranth (1.2 mg ßC) or VA (0.3 mg)	2 m	+
25. Bulux et al. (34), Guatamala	7-12 y	34	VA (1.0 mg), purified ßC (6 mg) + fat (10 g), or carrots (6 mg ßC)	20 d	0
26. de Pee et al. (35)	7-11 y	20-21	Vegetables (684 RE), fruit (509 RE), retinol-rich (556 RE), low carotene/low retinol (44 RE)	9 w	+

ßC-ß-carotene; VA-vitamin A; RE-retinol equivalent; mgnal-marginal

The impact of ß-carotene or ß-carotene-rich foods on vitamin A status

Although the rigor of the experimental designs in the 26 studies varied, only four did not show an improvement in vitamin A status after consumption of either purified ß-carotene or ß-carotene-rich foods.

Of the four that showed no effect, two studies included children who were overall not vitamin A deficient at the baseline: serum retinol was 33-34 µg/dL in the study by Bulux et al. in Guatemala (study 25) and in the Hussein and El Thomamy study in Egypt (study 8).

Of the remaining 22 studies:

- 16 showed an improvement in vitamin A status after consuming 0.8 to 19 mg ß-carotene as ß-carotene-rich foods (studies 1,2,3,4,5,6,7,11,15,16,18,19,20,21,24 and 26);
- 4 showed an improvement in vitamin A status after consuming 1.8 to 2.4 mg ß-carotene as red palm oil (studies 13,14,22 and 23);
- 4 showed an improvement in status after consuming 1.2 to 2.4 mg ß-carotene as purified ß-carotene (studies 3,4,12 and 19);
- 1 showed the vitamin A status of children who did not consume ß-carotene-rich foods worsened during the intervention; thus, ß-carotene-rich foods prevented vitamin A deficiency in the intervention group (study 17);

- 1 showed an improvement in vitamin A status after consuming 3.5 mg ß-carotene but not 1.6 mg ß-carotene from ß-carotene-rich foods (study 20). The baseline vitamin A status of the former group, however, was worse than the latter.

Yin et al's (study 17) and Solon et al's (study 18) studies used stable isotopes to determine vitamin A status, which is a more accurate assessment method than blood retinol levels.

Impact of ß-carotene versus vitamin A on vitamin A status

Among the studies with a positive control, a comparison can be made of the benefit of ß-carotene, be it purified or provided as foods, with vitamin A supplements. Any difference in vitamin A status between the ß-carotene and the vitamin A supplemented groups would be due to the efficiency of bioconversion and the presence of ß-carotene absorption enhancers and inhibitors. Of the 26 studies on children, nine included a positive control using vitamin A (studies 10,11,12,13,14,22,23,24 and 25) and one using retinol-rich food (study 26).

- 4 of the 10 studies compared red palm oil with vitamin A and palm oil was shown to have a benefit similar to vitamin A (studies 13,14,22 and 23);
- 1 study (study 25), showed no improvement in vitamin A status from either vitamin A or purified ß-carotene. As mentioned before, the children in this study were not deficient at the baseline;
- 1 study compared ß-carotene from vegetables and ß-carotene from fruit with retinol-rich food. Serum retinol improved in all three groups and the increase was larger in the retinol-rich group than the fruit and vegetable groups (study 26).

Of the remaining four studies that included comparisons between ß-carotene and vitamin A:

- Study 11 in Egypt, showed that ß-carotene-rich foods had a similar effect to a mega dose vitamin A supplement in improving vitamin A status;
- Study 12 in Senegal, showed that purified ß-carotene and vitamin A supplements similarly reduced the prevalence of abnormal eye cytology;
- Study 24 in India, showed that both ß-carotene and vitamin A improved vitamin A status but the improvement was greater after intervening with vitamin A than with ß-carotene;
- Study 10 in Indonesia, showed no effect on vitamin A status.

Impact of purified ß-carotene versus ß-carotene rich foods on vitamin A status

Purified ß-carotene can also be used as a positive control to evaluate the contribution of provitamin A foods to vitamin A status. Four of the 26 studies (studies 3,4,19,25) attempted to include purified ß-carotene as a control.

In the Devadas and Murthy study (Study 3), vitamin A status improved after the consumption of both amaranth and purified ß-carotene but they did not distinguish the effects of the amaranth from that of the purified ß-carotene.

The Ghana study (Study 19) found that vitamin A status improved after the consumption of ß-carotene-rich vegetables (± fat) and purified ß-carotene and the results are preliminary.

The Guatemala study (Study 25), as mentioned earlier, included children who were not deficient; thus, there would be little room for improvement in status.

Only one study, conducted in India (Study 4), which included vitamin A deficient children, has shown that ß-carotene-rich foods and purified ß-carotene improved vitamin A status to the same extent. These children were quite deficient in vitamin A having baseline retinol levels of 12-14 µg/dL.

Intervention studies among pregnant and lactating women

Six studies have been conducted among pregnant and lactating women (Table 6) (36-41) and their experimental designs have been more rigorous than those for children; all the studies have at least a negative control.

In Table 6, time refers to the duration of the regimen. For example, de Pee et al. (Study 27) gave the intervention 5 days/week for 12 weeks. Rice et al. (Study 28) gave one group a mega dose of vitamin A post partum and the other group a purified ß-carotene supplement daily for eight months. Effect refers to the intervention by group. For example, de Pee et al. (Study 27) showed a positive effect using purified ß-carotene but not with vegetables, while Yamini et al. (Study 29) showed a positive effect among both pregnant and lactating women.

Collectively, five of the six studies (Studies 28,29,30,31 and 32) showed that ß-carotene and/or ß-carotene-rich foods can improve vitamin A status and, more important, can reduce the clinical symptoms of a deficiency. Yamini et al (Study 29) found that vitamin A and purified ß-carotene improved the vitamin A status of pregnant and lactating women by 32% and 11%, respectively. Similarly, Christian et al. (Study 30) found that vitamin A and purified ß-carotene reduced the incidence of night blindness by 50-60% and 30-40% in pregnant and lactating women, respectively.

Wasantwisut et al. (Study 32) found that both serum and breast milk retinol levels increased in the intervention and control groups 12 weeks post intervention. The ß-carotene-rich foods group, however, consumed one-half to one-third less vitamin A from animal foods at baseline and during the intervention compared with the control groups. Serum and breast milk retinol levels increased the most in the purified ß-carotene group followed by the ß-carotene-rich foods group, and then the low ß-carotene control group. Isotope analysis to assess body vitamin A stores is in progress.

Canfield et al. (Study 31) found that retinol levels in Honduran mothers did not increase, but they did in their infants after consuming ß-carotene-rich fruits and vegetables and synthetic ß-carotene compared with the placebo.

Rice et al's Bangladesh study (Study 28) showed the

importance of the duration of an intervention. They found that it took nine months for a daily ß-carotene supplement to improve milk vitamin A levels, whereas it took two months to see an effect from a single high dose of vitamin A but the effect was not sustained; hence the brackets in the table for effect. The levels of both vitamin A and ß-carotene were not sufficient, however, to correct the underlying subclinical deficiency nor bring the infants to adequate status.

Only de Pee et al. have found that vitamin A status did not improve from ß-carotene-rich foods alone (Study 26). The women, however, were marginally vitamin A deficient and the dose of ß-carotene provided in this study was about 50% of that given in Honduras (Study 30).

TABLE 6
Studies with both a negative and positive control group among pregnant/lactating women

Study/Source/Site	µg/dL	Group	Intervention	Time	Effect
27. de Pee et al. (36), Indonesia	25	Preg	Purified ßC (3.5 mg)	5d/w/12 w	+
			Vegetables (3.5 mg ßC)		0
28. Rice et al. (37), Bangladesh	na	Lact (pp)	VA (60 mg)	Once	(+)
			Purified ßC (7.8 mg)	d/8 m	+
29. Yamini et al. (38), Nepal	na	Preg	VA (7 mg) or purified ßC	d/3.5+y	+
		Lact	(42 mg)		+
30. Christian et al. (39), Nepal	XN	Preg	VA (7 mg) or purified ßC	d/3.5+y	+
		Lact	(42 mg)		+
31. Canfield et al. (40), Honduras	31.3	Lact	ßC-rich foods (7.5 mg) or	3x/w/4 w	+
	16	Infants	purified ßC (7.5 mg)		+
32. Wasantwisut et al. (41), Thailand	na	Lact	ßC-rich foods (4.7 mg)	5d/w/	+
			or purified ßC (3.6 mg)	12w	+
					+

ßC-ß-carotene; VA-vitamin A; na-not available; pp-post partum;XN-night blindness

CONCLUSIONS

Based on the studies cited here, there is clearly a dearth of data from well-designed studies to argue that, except for red palm oil, ß-carotene-rich foods are effective in eliminating vitamin A deficiency. The evidence, however, leans toward ß-carotene-rich fruit and vegetable intake improving vitamin A status in deficient children and women. Vitamin A is more effective than ß-carotene in preventing vitamin A deficiency because there are many factors that affects the bioconversion of ß-carotene (4). Nevertheless, increasing the consumption of fruits and vegetables that are widely available in developing countries is a viable and sustainable approach to preventing vitamin A deficiency, especially where coverage of pharmaceutical supplements and vitamin A-fortified foods are limited.

The FAO RDA for vitamin A for children one to 10 years old is 400 RE (42), which is equivalent to about 3.4 mg ß-carotene. Data compiled by West and Poortvliet (43), showed that in develping countries:

- 100 g of uncooked or cooked carrots, which is equivalent to about one cup of grated carrots or two-thirds of a cup of diced carrots, contains between 1.6-64 mg ß-carotene;
- 100 g or just over one-half cup of cooked spinach contains between 2.5-5.8 mg. A similar amount of amaranth contains between 0.25-31.6 mg ß-carotene.
- 100 g or just over one-half cup of mango contains between 0.3-2 mg ß-carotene.

Thus, where varieties of ß-carotene-rich foods are available, manageable amounts of these foods can be eaten to meet requirements.

The challenge for programs is to encourage households to change their eating habits so that those most vulnerable to vitamin A deficiency have better access to both vitamin A-rich and ß-carotene-rich fruits and vegetables. At a minimum, this requires that such foods are available at a price that people can afford which, in turn, is dependent on climatic conditions and market infrastructure. In addition, the foods to promote must not be regarded as inferior foods and they must be palatable to the target groups.

REFERENCES

1. Olson J.A. 1992 Atwater Lecture. The irresistible fascination of carotenoids and vitamin A. Am J Clin Nutr 1993; 57: 833-9.
2. Rodriguez-Amaya D.B. Stability of carotenoids during the storage of foods. In: Charalambous G, editor. Shelf life studies

of foods and beverages: Chemical, biological, physical, and nutritional aspects. Amsterdam: Elsevier Science, 1993: 591-628.
3. WHO. Global prevalence of vitamin A deficiency. [WHO/NUT/95.3]. Geneva, 1995
4. de Pee S, West EC. Dietary carotenoids and their role in combating vitamin A deficiency: A review of the literature. Eur J Clin Nutr 1996; 50: S38-53.
5. Parker RS. Absorption, metabolism, and transport of carotenoids. FASEB J 1996; 10 (5): 542-51.
6. Food and Nutrition Board, National Research Council. Recommended dietary allowances. Fat soluble vitamins. National Academy Press, 1989, 78-92.
7. van Vliet T, Van Schaik F, Van Den Berg H, Schreurs WHP. Effect of vitamin A and ß-carotene intake on dioxygenase activity in the rat intestine. Ann N Y Acad Sci 1993; 691:220-2.
8. Solomons NW, Bulux J. Effects of nutritional status on carotene uptake and bioconversion. Ann NY Acad Sci 1993; 691:96-109.
9. Burri BJ, Neidlinger T, Clifford AJ. Serum ß-carotene concentration during carotenoid depletion is influenced by the body pool of vitamin A. FASEB J 1996; 1371:A238.
10. Pereira SM, Begum A. Studies in the prevalence of vitamin A deficiency. Ind J Med Res 1968; 56:362-9.
11. Lala VR, Reddy V. Absorption of ß-carotene from green leafy vegetables in undernourished children. Am J Clin Nutr, 1970; 23: 110-3.
12. Devadas RP, Murthy NK. Biological utilization of ß-carotene from amaranth and leaf protein in preschool children. World Rev Nutr Diet, 1978; 31: 159-61.
13. Devadas RP, Premakumari S, Subramanian G. Biological availability of ß-carotene from fresh and dried green leafy vegetables on preschool children. Ind J Nutr Diet 1978; 15: 335-40.
14. Jayarajan P, Reddy V, Mohanram M. Effect of dietary fat absorption of ß-carotene from green leafy vegetables in children. Ind J Med Res 1980; 71: 53-6.
15. Charoenkiatkul S, Valyasevi A, Tontisirin K. Dietary approaches to the prevention of vitamin A deficiency. Food Nutr Bull 1985; 7:72-6.
16. Mariath JGR, Lima MCC, Santos LMP. Vitamin A activity of buriti (*Mauritia vinifera* Mart) and its effectiveness in the treatment and prevention of xerophthalmia. Am J Clin Nutr, 1989; 49:849-953.
17. Hussein L, El-Tohamy M. Effect of supplementation with vitamin A or plant carotenes on plasma retinol levels among young Egyptian males. Int J Vit Nutr Res, 1989; 59:229-33.
18. Nasoetion A, Riyadi H, Baliwati YF. In: de Pee S, West CE. Dietary carotenoids and their role in combating vitamin A deficiency: A review of the literature. Eur J Clin Nutr 1996; 50: S38-53.
19. Muhilal, Karyadi D. A study on the bioavailability of vegetable carotenes and preformed vitamin A added to salt in preschool children. Paper presented at IVACG Meeting, Geneva, 1977.
20. Hussein L, El-Tohamy M. Vitamin A potency of carrot and spinach carotenes in human metabolic studies. Int J Vit Nutr Res 1990; 60:229-35.
21. Carlier C, Coste J, Etchepare M, Periquet B, Amedee-Manesme O. A randomised controlled trial to test equivalence between retinyl palmitate and beta carotene for vitamin A deficiency. Brit Med J 1994; 307:1107-10.
22. Rukmini C. Red palm oil to combat vitamin A deficiency in developing countries. Food Nutr Bull 1994; 15:126-9.
23. Manorama R, Brahmam GN, Rukmini C. Red palm oil as a source of beta-carotene for combating vitamin A deficiency. Plant Foods Hum Nutr 1996; 49:75-82.
24. Jalal, F, Nessheim MC, Agus Z, Sanjur D, Habicht JP. Serum retinol concentrations in children are affected by food sources of ß-carotene, fat intake, and anthelmintic drug treatment. Am J Clin Nutr 1998; 68:623-9.
25. Wadhwa A, Singh A, Mittal A, Sharma S. Dietary intervention to control vitamin A deficiency in seven- to twelve-year-old children. Food and Nutr Bull 1994; 15:53-6.
26. Yin S, Qin J, Gu SF, Xu QM, Zhao SF, Tang G, Russell RM. Green and yellow vegetables rich in provitamin A carotenoids can sustain vitamin A status of children. FASEB Abstract 1998; 2043-351.
27. Solon FS, Ribaya-Mercado JD, Pedecto CS, Cabal-Barza MA, Tang G, Russell RM, Solon MA. Improvement in vitamin A status of malnourished schoolchildren with fruit and vegetable intakes. FASEB Abstract 1998; 4873:841.
28. Takyi EEK, Owusu F. Bioavailability of carotenoids in leafy vegetables consumed by preschool children in Northern Ghana. IVACG Abstracts 1999:44.
29. Persson V, Gebre-Medhin M, Ahmed F, Greiner T. Effect of feeding with DGLV on vitamin A status in ascaris-free Bangladeshi school children. IVACG Abstracts p. 42, 1999.
30. Roels OA, Trout M, Dujacquier R. Carotene balances in boys in Ruanda where vitamin A deficiency is prevalent. J Nutr 1958; 65:115-27.
31. Roels OA, Djaeni S, Trout ME et al. The effect of protein and fat supplementation on vitamin A-deficient Indonesian children. Am J Clin Nutr 1963; 12:380-7.
32. Lian OK, Tie LT, Rose CS, Prawiranegara DD, György P. Red palm oil in the prevention of vitamin A deficiency. A trial on preschool children in Indonesia. Am J Clin Nutr 1976; 20:1267-74.
33. Devadas RP, Saroja S, Murthy NK. Availability of ß-carotene from papaya fruit and amaranth in preschool children. Ind. J Nutr Diet 1980; 17:41-4.
34. Bulux J, Quan de Serrano J, Giuliano et al. Plasma response of children to short-term chronic ß-carotene supplementation. Am J Clin Nutr 1994; 59:1369-75.
35. De Pee S, West CE, Permaesih D, Martuti S, Muhilal, Hautvast GAJ. Orange fruit is more effective than are dark-green leafy vegetables in increasing serum concentrations of retinol and ß-carotene in schoolchildren in Indonesia. Am J Clin Nutr 1998; 68:1058-67.
36. De Pee S, West CE, Muhilal, Karyadi D, Hautvast GAJ. Lack of improvement in vitamin A status with increased consumption of dark-green leafy vegetables. Lancet 1995; 346:75-81.
37. Rice AL, Stoltzfus RJ, de Francisco A, Chakraborty J, Kjolhede CL, Wahed, MA. Maternal vitamin A or ß-carotene supplementation on lactating Bangladeshi women benefits mothers and infants but does not prevent subclinical deficiency J Nutr 1999; 129:265-356.

38. Yamini S, Zhou L, Wu LSE, Yang D, Dreyfuss ML, West KP. Impact of ß-carotene supplementation on circulating levels of retinol, tocopherols and other carotenoids in Nepalese women. FASEB Abstract 1998; 1244:213.
39. Christian P, West KP, Khatry SK et al. Night blindness of pregnancy in rural Nepal - nutritional and health risks. Int J Epid, 1998; 27:231-7.
40. Canfield LM, Alger J, de Kaminski RG, Liu, Y. Increasing ß-carotene intake of the lactating mother enhances the vitamin A status of the mother-infant pair. IVACG Abstracts 1999:43.
41. Wasantwisut E, Sungpuag P, Viriyapanich T et al. Impact of dietary vitamin A interventions on biochemical indicators in Thai lactating women. IVACG Abstracts 1999:43.
42. FAO/WHO. Requirements for vitamin A, iron, folate and vitamin B12. Report of a joint FAP/WHO expert consultation. FAO Food Nutr Series no 23. Rome: FAO, 1988.
43. West CE, Poortvliet EJ. The carotenoid content of foods with special reference to developing countries. Washington DC: USAID-VITAL, 1993.

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Enrichment of the diet with synthetic and natural sources of provitamin A

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SUMMARY. The use of available food rich in provitamin A and retinol as well as fortification of local food are known to result in adequate vitamin A status. In Brazil, several regional foods are known to be good sources of provitamin A such as buriti, several palm oils, mango and others. Improving the consumption of these locally available natural sources of provitamin and vitamin A would cover the needs of the vulnerable population. At the same time fortification of industrialized foods with natural and/or synthetic forms of provitamin A could speed up and fill the gap between requirement and low intake of this vitamin in many parts of the country. This approach has been considered by many as the most effective intervention program to prevent micronutrient deficiencies in developing countries. Our previous studies on the subject have shown that cooking vegetable oil, mainly soybean oil, is a very good alternative vehicle to be fortified and supply vitamin A to the population. Lately we have also enriched the same soybean oil with ß-carotene. Addition of this provitamin A to the oil showed it to be stable when heated at cooking and frying temperatures (retention of 92.4±6.7% and 65.4±8.6%, respectively). When rat or human food was prepared with carotene-enriched cooking oil, its bioavailability in experimental animals and absorption in humans were shown to be adequate. An alternative for Brazil, besides adding chemical forms of the vitamin to the cooking oil, would be to mix available carotene-rich palm oil to the soybean oil. There are already regional uses of carotenoid-rich palm oils in the preparation of local dishes in some parts of Brazil and this would facilitate its acceptance by the population. Enrichment of common foods in Brazil, such as soybean oil, with chemical forms of ß-carotene or mixing rich sources of provitamin A can be a good alternative to improve the intake of vitamin A by the Brazilian population.

Key words: ß-carotene, micronutient fortification, soybean oil fortification, Brazil.

RESUMO. Enriquecimento da dieta con pró-vitamina A usando fontes naturais e sintéticas. O uso de alimentos localmente disponíveis, ricos em pró-vitamina A e retinol, bem como a fortificação de alimentos, apresenta resultados conhecidos na melhora do estado nutricioal em relação a esse nutriente. No Brasil, diversos alimentos regionais são conhecidos como boas fontes de pró-vitamina A tais como o buriti, azeite de dendê, manga o outros. O aumento do consumo desses alimentos poderia suprir as necessidades de vitamina A da população vulnerável. Ao mesmo tempo, a fortificação de alimentos industrializados com forma sintética ou natural de pró-vitamina A poderia contribuir para uma adequação mais rápida da quantidade de vitamina A ingerida em muitas partes de nosso país. O enriquecimento de alimentos tem sido considerado por muitos como método mais efetivo para prevenir a deficiência de micronutrientes em países en desenvolvimento. Nossos estudos prévios sobre esse assunto têm mostrado que o óleo vegetal, especialmente o de soja, é um bom veículo alternativo para a fortificação com vitamina A. Mais tarde, nós também enriquecemos o óleo de soja com ß-caroteno. Nossos estudos mostraram que o ß-caroteno é bastante estável quando aquecido a temperatura de cozimento e fritura (retenção de 92,4±6,7 e 65,4±8,6%, respectivamente). O cozimento de alimentos preparados com óleo enriquecido não alterou significativamente a biodisponibilidade do ß caroteno sintético em ratos nem sua absorção em humanos. O enriquecimento do óleo de soja com óleos de palma naturalmente ricos em pró-vitamina A pode ser uma alternativa para o Brasil. Oleos de palma são utilizados no preparo de pratos regionais em algumas partes do país, e isso poderia facilitar a aceitação pela população. O enriquecimento de alimentos, como o óleo de soja, usualmente consumidos no Brasil, con ß-caroteno sintético ou através da mistura de fontes naturais de pró-vitamina A pode ser uma boa alterenativa para aumentar a ingestão de vitamina A pela nossa população.

Palavras chave: ß caroteno, fortificação com micronutientes, fortificação do óleo de soja, Brasil.

INTRODUCTION

Studies on the biological and functional effects of carotenoids are lately growing and there is a great interest on their results. The provitamin A activity and availability of several carotenoids are important, considering that almost 60% of dietary vitamin A is supplied as provitamin A (1), reaching 85% in developing countries because of the high cost of animal foods (2).

The best sources of carotenoids are green leafy vegetables, carrots, sweet potatoes, pumpkins, yellow and orange tropical fruits and several palm fruits, quite common in Brazil and other tropical countries (3).

For a food to be considered a good source of provitamin A, it is necessary to have not only a large amount of the carotenoids, but also a high bioavailability. The presence of dietary fiber

and fat has influence on their absorption (3,4). The effect of industrial processing on their utilization is also known, increasing or decreasing it (3).

The bioavailability of carotenoids as a source of vitamin A in humans have been analyzed in several studies (Table 1). As vitamin A deficiency is a great public health problem in several parts of the world, it is necessary to know the utilization and effectiveness of different foods on the prevention of this micronutrient problem and to improve the consumption of vitamin A-rich foods, mainly by children.

TABLE 1
Effect of provitamin A on the improvement of nutritional status of children living in high prevalence areas of vitamin A deficiency

Food	Author	Country	Effects observed
Buriti (sweet of buriti)	Mariath et al (5)	Brazil	Restoration of liver reserves - relative dose response. Regression of clinical xerophthalmia
Spinach (boiled)	Hussein & El Tohamy (6)	Egypt	Increase in plasma retinol levels
Carrots (grated)	Hussein & El Tohamy (7)	Egypt	Increase in plasma retinol levels
Palm oil	Lian et al. (7)	Indonesia	Increase in serum vitamin A levels Decrease in prevalence of xerophthalmia
Palm oil	Rukmini C. (8)	India	Increase in serum vitamin A levels Restoration of liver reserves - modified relative dose response
Dark-green leafy vegetables	Charoenkiatkul et al. (9)	Thailand	Increase in serum vitamin A levels
Carrot, papaya and coriander-mint chutney	Wadhwa et al. (10)	India	Increase in serum vitamin A levels

The ACC/SCN Consultative Group (11) suggested four activities to improve consumption of vitamin A from local diets in risk populations: (a) nutrition education or communication, using social marketing approach, to improve practices related to the consumption of available vitamin A-rich food sources; (b) horticultural interventions and home food gardens to increase availability of vitamin A-rich foods; (c) economic/food policies affecting availability, price and effective demand for vitamin A-rich foods; (d) technological advances concerning food preservation, plant breeding, etc.

There is no doubt that improving consumption of vitamin A-rich foods is a good and inexpensive approach to prevent this micronutrient deficiency, when these foods are available and consumed locally. At the same time these foods are, sometimes, sources of other vitamins and minerals. When food behaviour changes are needed, the problems is much more complicated. Success of this intervention is linked to several other socio-economic and cultural factors that should be solved simultaneously.

On the other hand, enrichment of industrialized food is considered by many experts as the most effective method to prevent micronutrient deficiencies, including vitamin A (2).

Several food products have been used to increase the intake of vitamin A in different parts of the world. Vehicles such as sugar, tea, cereals, monosodium glutamate were tried and some of them are still currently used (12-15).

The literature is limited in relation to food fortification with carotenoids as a source of vitamin A. It has a large industrial use, mainly as food improvers and colorants (16,17).

In Brazil, several pasta manufacturers add ß-carotene in the amount of 2,000 to 4,000 IU/kg of their product. There is a high consumption of macaroni in Brazil and its consumption is considerable among low socio-economic level people. It is inexpensive and can be a good alternative for enrichment.

Pereira et al. (16) verified the amount of total carotenoid and ß-carotene in 41 samples of fortified spaghetti from the six largest plants in Brazil and found amounts as specified in the labels, in the greatest part of the samples. They also cooked several samples and measured the amount of ß-carotene before and after heating. A good retention of ß carotene was found after cooking. Their conclusion was that the fortification of dried pasta could be valid.

In Brazil, Chile, Colombia, Mexico, Honduras, El Salvador, Guatemala, Panamá, Equador and Peru, the addition of vitamin A to margarine is compulsory. ß-carotene is sometimes added to the product, but only as a coloring agent. Our laws do not specify the percent of vitamin A that may be present as ß-carotene. Rader et al. (17) analysed 19 margarine and similiar products sold in Washington in relation to the amount of retinyl ester and ß-carotene. Except in one product, ß-carotene

was equivalent to 20-40% of the total vitamin A content.

We have been studying for the last 6-8 years the fortification of cooking vegetable oils, mainly soybean oil, as a carrier to supply vitamin A to the population in need (18-20). The product is consumed daily all over the country by the population, including low socio-economic groups. Soybean oil has many characteristics which make it a very attractive and useful carrier of oil-soluble products such as vitamin A and carotene. Brazil is the second largest world producer of soybean. The crop is industrially processed in a few large industrial plants, from where it is distributed all over the country. There is good industrial quality control and the oil is rich in energy, unsaturated fatty acids and vitamin E. It favors the homogenization of fat-soluble vitamins and their absorption.

Our present studies with the fortificatin of cooking soybean oil with ß-carotene (21) showed that after cooking (100°C-20 min.) ß-carotene retention was 92.3±6.7% and after frying (170°C/3 times) 65.4±8.5% (Table 2).

TABLE 2
Stability of ß-carotene in fortified soybean oil after heat treatment (21)

Sample	Retention of ß-carotene (%) 100°C 20 min	170°C three times
1	99.7	48.7
2	89.5	63.6
3	80.1	60.7
4	92.0	70.1
5	90.0	71.5
6	97.6	72.4
7	97.6	70.6
Mean ± SD	92.3±6.7a	65.4±8.5b

Values with different letters in the same line are significantly different at $p < 0.05$ (Tukey's test)

Evaluation of bioavailability of the carotenoid added to oil before and after heating, were carried out in rats (21). It was found that the amount of retinol stored in the liver of animals receiving diets with 4 RE/g of ß-carotene, when heated at 100°C during 20 minutes, did not show statistical difference from the group which received the same amount of carotene in unheated oil. The group which received the diet with the enriched oil heated at 170°C three times had reduced liver levels of vitamin A, but had values similar to those of the control group that received unheated oil, having 2 RE/g (Table 3). Higher temperature and succesive heating seem to reduce levels of stored vitamin A, but reasonable levels are still kept.

In our last paper on the subject (22), the absorption in humans of ß-carotene added to soybean oil was measured before and after heating, when the oil was used to cook Brazilian food. Sixteen healthy adults, males and females, received on the first trial day the same meal including rice cooked with carotene-enriched oil or unenriched oil. The meal consisted of rice, beans and meat in the same proportion and quantity. Blood was collected five fasting times (1,2,3,7,11 days of study) and plasma carotene was measured. Absorption was calculated by the peak rise and the area under the curve (Figure 1 and Table 4). Results showed that the ß-carotene added to the soybean oil was well absorbed with or without heating.

TABLE 3
Plasma and liver total vitamin A of rats fed diets containing ß-carotene-fortified soybean oil with and without heat treatment (21)

	Plasma (µmol/L)	Liver (µmol/g)
Diet 1-2 RE/g, no heat	1.29±0.23a	0.47±0.09a
Diet 2-4 RE/g, no heat	1.19±0.39a	0.64±0.08b
Diet 3-8 RE/g, no heat	1.23±0.19a	0.97±0.14c
Diet 4-4 RE/g, heat 100°C	1.11±0.32a	0.72±0.06b
Diet 5-4 RE/g, heat 170°C	1.12±0.28a	0.45±0.04a

Values are expressed as means ± SD. Values with different letters in the same column are significantly different at $p < 0.05$ (Tukey's test)

FIGURE 1
Plasma fasting levels of ß-carotene after rice intake with fortified soybean oil, added during or after cooking

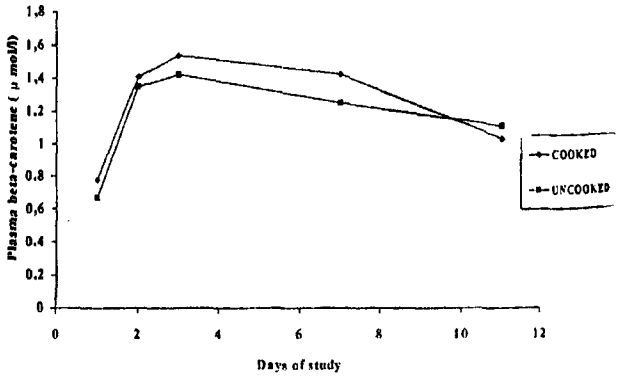

TABLE 4
Efect of heat treatment during rice cooking with ß-carotene-fortified soybean oil and its impact on postprandial absorption of ß-carotene in human subjects. Data as X ± SEM (22)

	Area under curve (µmol/h/L) Cooked	Uncooked	Peak rise (µmol/l) Cooked	Uncooked
Men (n=6)	1.69±0.260	1.33±0.232	0.66±0.097	0.49±0.062
Women (n=10)	2.25±0.358	2.86±0.584	1.04±0.117	1.14±0.238
Total (n=16)	2.04±0.248	2.27±0.415	0.90±0.091	0.90±0.168

The possibility of enriching cooking soybean oil with other provitamin A-rich natural oils available in Brazil, as

buriti and palm oil, is a good alternative for Brazil because the amount of provitamin is quite high and the Brazilians are used to their taste and color. It can be a good local alternative to supply carotenoids to the population. It confers on the oil a nice orange color that can help foster its acceptance. Further studies should be carried out on this subject.

REFERENCES

1. Simpson KL. Relative value of carotenoids as precursors of vitamin A. Proc Nutr Soc 1983;42:7-17.
2. McLaren DS, Frigg M. Sight and life malnutrition vitamin A deficiency disorders (VADD). Basel: Task Force Sight and Life, 1997.
3. Rodríguez-Amaya DR. Carotenoids and food preparation: The retention of provitamin A carotenoids in prepared, processed and stored foods. Arlington: Opportunities for Micronutrient Intervention (OMNI), 1997.
4. Rock CL, Servendseid ME. Plasma ß-carotene response in human after meals supplemented with dietary pectin. Am J Clin Nutr 1992;55:96-9.
5. Mariath JGR, Lima MCC, Santos LMP. Vitamin A activity of buriti (*Mauritia vinifera* Mart.) and its effectiveness in the treatment and prevention of xerophthalmia. Am J Clin Nutr 1989;49:849-53.
6. Hessein L, El-Tohamy M. Effect of supplementation with vitamin A or plant carotenes on plasma retinol levels among young Egyptian males. Int J Vit Nutr Res 1988;59:229-33.
7. Lian OK, Tie LT, Rose CS, Prawiranegara DD, Gyögy P. Red palm oil in the prevention of vitamin A deficiency. Am J Clin Nutr 1967;20:1267-74.
8. Rukmini C. Red palm oil to combat vitamin A deficiency in developing countries. Food Nutr Bull 1994;15:126-9.
9. Charoenkiatkul S, Valyasevi A, Tontisirink. Dietary approaches to the prevention of vitamin A deficiency. Food Nutr Bull 1985;7:72-6.
10. Wadhwa A, Singh AM, Sharma S. Dietary intervention to control vitamin A deficiency in seven-to twelve-year-old children. Food Nutr Bull 1994;15:53-6.
11. ACC/SCN Consultative Group. Controlling vitamin A deficiency. ACC/SCN State-of-the-Art Series, Nutrition Policy Discussion Paper nº 14, Ottawa: United Nations, 1994.
12. Arroyave G, Mejia LA, Aguilar JR. The effect of vitamin A fortification of sugar on the serum levels of preschool Guatemalan children: a longitudinal evaluation. Am J Clin Nutr 1981;34:41-9.
13. Muhilal A, Murdiana I, Azis S, Saidin S, Jahari AB, Karyadi D. Vitamin A fortified monossodium glutamate and vitamin A status: a controled field trial. Am J Clin Nutr 1989;48:1265-70.
14. Brooke CL, Cort WM. Vitamin A fortification of tea. Food Technol 1972;26:50-2.
15. Murphy P, Smith B, Hauck C, Connor KO. Stabilization of vitamin A in a synthetic rice premix. J Food Sci 1992;57:437-9.
16. Pereira MR, Amaya-Farfan J, Rodriguez-Amaya DB. ß-carotene content of Brazilian fortified pasta. Food control 1999;10:81-5.
17. Rader YI, Weaver CM, Patrascu L, Ali H, Anyal G. α-Tocopherol, total vitamin A and total fat in margarine and margarine-like products. Food Chem 1997;58:373-9.
18. Fãvaro RMD, Ferreira JF, Desai ID, Dutra de Oliveira JE. Studies on fortificatin of refined soybean oil with all trans retinyl palmitate in Brazil: stability during cooking and storage. J Food Comp Anal 1991;4:237-44.
19. Fávaro RMD, Miyasaaka CK, Desai ID, Dutra de Oliveira JE. Evaluation of the effect of heat treatment on the biological value of vitamin A fortified soybean oil. Nutr Res 1992;12:1357-63.
20. Dutra de Oliveira JE, Desai ID, Fávaro RMD, Ferreira JF. Effect of heat treatment during cooking on the biological value of vitamin A fortified soybean oil in human. Int J Food Sci Nutr 1994;45:203-7.
21. Dutra de Oliveira JE, Fávaro RMD, Junqueira Franco MVM, Carvalho CG, Jordão AA Jr, Vannucchi H. Effect of heat treatment on the biological value of ß-carotene added to soybean cooking oil in rats. Int J Food Sci Nutr 1998;49:205-9.
22. Dutra de Oliveira JE, Fávaro RMD, Leonardo IR, Jordão AA Jr, Vannucchi H. Absortion by humans of beta-carotene from fortified soybean oil added to rice: Effect of heat treatment. J Amer Coll Nutr 1998;17:361-5.

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Changes in carotenoids during processing and storage of foods

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SUMMARY. Being highly unsaturated, carotenoids are susceptible to isomerization and oxidation during processing and storage of foods. Isomerization of *trans*-carotenoids to *cis*-carotenoids, promoted by contact with acids, heat treatment and exposure to light, diminishes the color and the vitamin A activity of carotenoids. The major cause of carotenoid loss, however, is enzymatic and non-enzymatic oxidation, which depends on the availability of oxygen and the carotenoid structure. It is stimulated by light, heat, some metals, enzymes and peroxides and is inhibited by antioxidants. Data on percentage losses of carotenoids during food processing and storage are somewhat conflicting, but carotenoid degradation is known to increase with the destruction of the food cellular structure, increase of surface area or porosity, length and severity of the processing conditions, storage time and temperature, transmission of light and permeability to O_2 of the packaging. Contrary to lipid oxidation, for which the mechanim is well established, the oxidation of carotenoids is not well understood. It involves initially epoxidation, formation of apocarotenoids and hydroxylation. Subsequent fragmentations presumably result in a series of compounds of low molecular masses. Completely losing its color and biological activities, the carotenoids give rise to volatile compounds which contribute to the aroma/flavor, desirable in tea and wine and undesirable in dehydrated carrot. Processing can also influence the bioavailability of carotenoids, a topic that is currently of great interest.

Key words: Carotenoids, processing, storage, isomerization, oxidation, degradation.

RESUMEN. Cambios en los carotenoides durante el procesamiento y almacenamiento de alimentos. Por ser altamente insaturados, los carotenoides son susceptibles a la isomerización y oxidación durante el procesamiento de los alimentos. La isomerización de *trans*- para *cis*-carotenoides, promovida por el contacto con ácidos, tratamiento con calor y exposición a la luz, disminuye el color y la actividad de vitamina A de los carotenoides. El principal causante de las pérdidas, sin embargo, es la oxidación por vías enzimáticas y no enzimáticas, la cual depende de la disponibilidad de oxigeno y la estructura del carotenoide. Esta es estimulada por la luz, el calor, ciertos metales, enzimas y peróxidos y es inhibida por anti-oxidantes. Si bien los datos sobre pérdidas porcentuales, durante el procesamiento y almacenamiento, son un tanto conflictivas, no hay duda de que la degradación aumenta con el grado de destrucción de las estructuras celulares, el incremento del área superficial o porosidad, tiempo y severidad de las condiciones del proceso, duración y temperatura del almacenamiento, transparencia a la luz y permeabilidad del embalaje al O_2. En contraste con la oxidación en lípidos, donde el mecanismo se encuentra bien definido, la oxidación de carotenoides no está elucidada. Esta comienza con epoxidación, formación de apocarotenoides e hidroxilación. Fragmentaciones subsecuentes presumiblemente llevan a una serie de compuestos de pequeña masa molecular. Después de perder su color y sus actividades biológicas, los carotenoides dan origen a compuestos que contribuyen al aroma/sabor, los cuales pueden ser deseables como en té y vino, pero indeseables en productos como zanahoria deshidratada. El procesamiento también puede influenciar la biodisponibilidad de los carotenoides, tema actualmente de gran interés.

Palabras clave: Carotenoides, procesamiento, almacenamiento, isomerización, oxidación, degradación.

INTRODUCTION

Processing and storage of foods have become integral parts of the modern-day food chain. Seasonal produce are processed during peak harvest, diminishing losses and making the products available all year round. Moreover, processing seasonal and perennial foods permits transportation of these foodstuffs to places far removed from the site of production.

Processing, however, can cause degradation of labile nutrients, biologically active compounds and substances important to food quality, such as colorants. Being highly unsaturated, carotenoids, for example, are prone to isomerization and oxidation, resulting in loss of color and biological activity. Thus, necessary measures will have to be taken to insure their retention during processing. Although industrial processing is more often focalized, losses on home preparation can also be, at times even more, considerable.

Literature on carotenoid retention during processing and storage of food is quite voluminous. However, published results are difficult to interpret because of the following reasons: (a) processing and storage conditions are not or are only partially described; (b) different foods are processed differently, making comparisons of processing methods difficult; (c) different conditions (e.g. time and temperature)

are used for the same method of processing; (d) the procedure followed for calculating losses is not specified; and (e) no correction is made for weight changes during processing and the usually greater efficiency with which carotenoids are extracted from processed (except for dehydrated products) compared to raw samples during analysis (1). Additionally, in most papers total carotenoid or carotene content was measured. In the present article, emphasis will be given to work involving determination of individual carotenoids. An inherent problem that cannot be overlooked is the possibility of isomerization and oxidation of carotenoids taking place during analysis and/or during storage of samples prior to analysis, these reactions being erroneously attributed to the processing or storage of food. Nevertheless, some conclusions can be drawn (1):

1. The tropical climate of many poor areas of the world enhances biosynthesis of carotenoids, increasing their concentrations during ripening/maturing of fruits and vegetables. On the other hand, this same ambient condition may hasten destruction of carotenoids during post-harvest handling and storage.
2. Carotenoid biosynthesis may continue in fruits, fruit vegetables and root crops, even after harvest, provided these plant materials are kept intact and not treated in any way that would inactivate the enzymes responsible for carotenogenesis. In leaves and other vegetables, post-harvest degradation of carotenoids appears to prevail, especially at high storage temperature and under conditions that favor wilting.
3. Carotenoids are naturally protected in plant tissues; cutting of fruits and vegetables into small pieces or maceration increases exposure to oxygen and brings together carotenoids and enzymes, which catalyze carotenoid oxidation.
4. The stability of carotenoids differs in different foods, even when the same processing and storage conditions are used. Carotenoids per se have different susceptibilities to degradation. Thus, optimum conditions for carotenoid retention during preparation/processing differ from one food to another.
5. The major cause of carotenoid destruction during processing and storage of foods is enzymatic or non-enzymatic oxidation. Isomerization of *trans*-carotenoids to the *cis*-isomers, particularly during heat treatment, also lessens the color and the vitamin A value of foods, but not to the same extent as oxidation. Enzymatic degradation of carotenoids may be a more serious problem than thermal decompositon in many foods.
6. Reported increases in carotenoid content during cooking or thermal processing are not likely to be true increases but are artifacts of the analytical procedure, due to loss of carotenoids in fresh samples because of enzymatic activity, greater extractability of carotenoids from processed samples, and unaccounted loss of water and leaching of soluble solids.
7. In home preparation, losses of carotenoids generally increase in the following order: microwaving < steaming < boiling < sautéing. Deep-frying, prolonged cooking, combination of several preparation and cooking methods, baking and pickling all result in substantial losses of carotenoids.
8. Whatever the processing method chosen, retention of carotenoid decreases with longer processing time, higher processing temperature and cutting or puréeing of the food. Reducing processing time and temperature, and the time lag between peeling, cutting or puréeing and processing improve retention significantly. High temperature, short-time processing is a good alternative.
9. The heat treatment in blanching may provoke some losses of carotenoids, but the inactivation of oxidative enzymes will prevent further and greater losses during holding before thermal processing, slow processing and storage.
10. Freezing (especially quick-freezing) and frozen storage generally preserve the carotenoids.
11. Peeling and juicing result in substantial losses of carotenoids, often surpassing those of heat treatment.
12. Traditional sun-drying, although the cheapest and most accessible means of food preservation in poor regions, causes considerable carotenoid destruction. Drying in a solar dryer, even of simple and inexpensive design, can appreciably reduce losses. Protecting the food from direct sunlight also has a positive effect.
13. Natural or added antioxidant and sulfiting may reduce carotenoid degradation.
14. Exclusion of oxygen (e.g. through vacuum or hot filling, oxygen-impermeable packaging, inert atmosphere), protection from light and low temperature diminish carotenoid decompositon during storage.

Alteration or losses of carotenoids during processing and storage therefore occur through physical removal, geometrical isomerization and enzymatic or non-enzymatic oxidation.

Freezing and frozen storage

Recent years have seen commercialization throughout Brazil of frozen fruit pulps, from tropical fruits hitherto available only in the producing Northeastern states. Used for the preparation of juices, these frozen pulps are generally processed by small industries. In frozen (-18°C) *Eugenia uniflora* pulp, ß-cryptoxanthin, γ-carotene and lycopene were considerably reduced (Table 1) (2). The product was not subjected to thermal treatment, the losses being apparently due to the removal of the peel during pulping. The carotenoids affected were those commonly concentrated in the peel. Carotenoids are found at higher levels in the peel than in the pulp of most carotenogenic fruits (3,4).

TABLE 1
Carotenoid composition (μg/g)* of fresh fruit and frozen *Eugenia uniflora* pulp

Carotenoid	Fresh fruit	Frozen pulp
Phytofluene	12a	11a
ß-Carotene	8a	7a
ζ-Carotene	4a	3a
ß-Cryptoxanthin	45a	12b
γ-Carotene	50a	15b
Lycopene	72a	26b
Rubixanthin	22a	22a
Total	215a	99b

*Means and standard deviations of 6 determinations. Values in the same row bearing different letters are significantly different ($p \leq 0.05$).
Reference: Cavalcante and Rodriguez-Amaya (2).

Further substantial reduction of the carotenoids of *E. uniflora* occurred during the first two months of storage at -15°C, stabilizing thereafter. Since the pulp was unblanched, enzymatic oxidation was the probable cause of these losses. The extent of loss was much greater than that usually seen in thermally processed products. Moreover, carotenoid decomposition in the latter products is usually insignificant during the first several months, increasing rapidly when it ensues.

The ß-carotene and lutein contents of unblanched and blanched (100°C, 4 min) chopped green beans and intact Padrón pepper, all frozen at -22°C, were monitored over 12 months (5). Both pigments decreased considerably during the first month in green beans packed in manually sealed and vacuum-sealed polyethylene bags. Lutein stabilized during the next 11 months; ß-carotene decreased further in the second month but stabilized thereafter. The smaller overall decrease of ß-carotene in blanched beans was attributed to deactivation of lipoxygenase. Reduction of lutein was roughly the same for blanched and unblanched beans. In contrast, the ß-carotene and lutein levels in the frozen pepper fluctuated around more or less constant values over the 12 months. Wu et al. (6) found no change in the ß-carotene content of green beans and broccoli during U.S. retail market simulation and frozen storage (blanched) at -20°C for 16 weeks.

Papaya slices without previous treatment were vacuum-packed in plastic bags and frozen in air-blast freezer operating at -40°C. The bags were left in the freezer until the center of the slices reached -24°C and then stored at -18°C for 12 months (7). The carotenoid content decreased significantly, the reduction being markedly higher in the female papaya slices than the hermaphrodite papaya slices (Table 2). The difference was attributed to greater enzymatic activity in the female papaya slices.

The influence of packaging materials with high, medium and low oxygen transmission rates on astaxanthin retention in rainbow trout fillets, containing three different levels of astaxanthin, during dark or illuminated frozen storage (-18°C) was studied (8). Samples were analyzed after 17, 29 and 36 weeks of frozen storage. Packaging material had significant effect on astaxanthin retention in the fillets while light or initial astaxanthin level did not have a significant influence.

TABLE 2
Carotenoid composition (μg/g)* of fresh and frozen papaya slices (stored for 12 months at -18°C) cultivar Sunrise

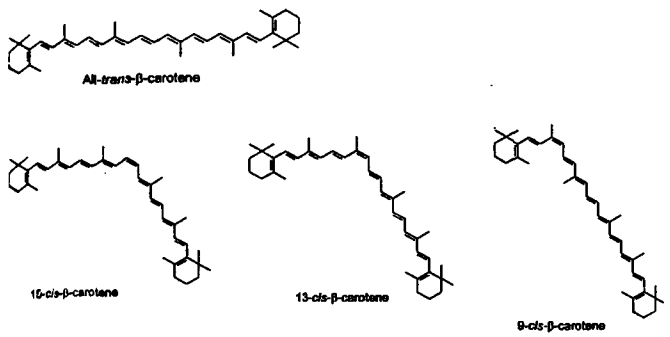
Carotenoid	Female/Hermaphrodite	
	Fresh	Frozen
Zeaxanthin	0.47/0.44	0.22/0.35
Cryptoflavin	0.42/0.16	-/0.10
ß-Cryptoxanthin	6.1/4.4	1.8/3.0
Lutein ester	0.70/0.32	0.25/0.33
Lycopene	19/20**	4.8/13
Neolycopene A	1.4/-	1.8/3.9
ß-Carotene-5,6-epoxide ester + ß-carotene	1.7/1.2	0.64/1.0
9-*Cis* or 9'-*cis*-cryptoxanthin ester	1.2/1.2	0.57/1.0
ß-Cryptoxanthin-5,6-epoxide ester	2.0/1.3	0.69/1.1
ß-Cryptoxanthin ester	3.9/2.4	1.8/2.6
ß-Cryptoxanthin ester	0.74/0.40	0.51/0.43
ß-Cryptoxanthin ester	- / -	0.19/0.13
Total	38/31	13/26

*Means of two determinations. Concentrations are reported as ß-carotene equivalents, except for zeaxanthin, ß-cryptoxanthin, lycopene and neolycopene A.
**Included neolycopene A
Reference: Cano et al. (7).

Thermal processing and storage

Occurrence of *trans* to *cis*-isomerization as a consequence of thermal processing has been shown by several authors. The major *cis* isomers of ß-carotene detected are shown in Figure 1. In a recent work, in which a polymeric C_{30} column was used for the separation of the isomers, a 10-39% increase in the percentage of total *cis*-isomers of provitamin A carotenoids in several fruits and vegetables was observed (Table 3) (9). Canning of sweet potato caused the largest increase, followed by processing of carrot, tomato juice, collard, tomato, spinach, peach and orange juice. The principal *cis*-isomers in processed red, yellow and orange fruits and vegetables were 13 *cis*-(and 13'-*cis*-), although 9-*cis*- and 15-*cis*-isomers were also detected. In processed green vegetables (in which ß-carotene was the only provitamin A carotenoid detected), 9-*cis*-ß-carotene predominated, followed by 13-*cis*-ß-carotene, an unidentified *cis*-isomer and 15-*cis*-ß-carotene.

FIGURE 1
Common geometrical isomers of ß-carotene



The isomerization pattern reported by Lessin et al. was also observed in earlier studies, the 13-*cis*- being the major *cis*-isomer in processed fruits and vegetables, except in processed green vegetables in which the 9-*cis*- prevailed (10-12).

In sweet potatoes, heat induced formation of 13-*cis*-ß-carotene in the different thermal treatments investigated, the quantity formed being related to the severity and length of processing (Table 4) (12). Only canned sweet potato contained appreciable amount of the 9-*cis*-isomer. Carotene content was reduced 20% on canning, 21% on dehydration, 23% on microwaving and 31% on baking. Apparent increases on blanching (4-12%) and puréeing (10%) were attributed to an enhanced extraction efficiency of heat-treated samples.

TABLE 3
Quantitative distribution of provitamin A carotenoid isomers (µg/g dry wt)* in fresh and processed fruits and vegetables

Product	ß-Carotene					α-Carotene					ß-Cryptoxanthin		
	all-*trans*	9-*cis*	13-*cis*	15-*cis*	other *cis*	all-*trans*	9-*cis*	13-*cis*	13'-*cis*	other *cis*	all-*trans*	13/13'-*cis*	15-*cis*
Carrot													
fresh	534					373							
canned	420	33	90	30		291	6.1	91	56	37			
Collard													
fresh	206	33	16	8.5	11								
canned	230	128	19	9.2	24								
Orange juice													
fresh	2.2	tr	0.4	tr		1.9	tr	0.2	0.1		2.5	0.2	tr
pasteurized	1.5	tr	0.3	tr		1.3	tr	0.1	0.1		1.3	0.2	tr
Peach													
fresh	2.2	0.3	0.5	tr							0.3	0.1	0.1
canned	0.9	0.2	0.4	tr							0.2	0.1	tr
Spinach													
fresh	312	39	24	tr	22								
canned	310	97	29	15	23								
Sweet potato													
fresh	256												
canned	191	25	77	19									
Tomato													
fresh	71	4.8	5.8										
canned	49	5.5	12	4.8									
juice	40	4.5	10	4.8									

*Means of two lots, except for orange juice, which had only one lot. Tr- trace.
Reference: Lessin et al. (9).

Cis-isomers also increased during heating of carrot juice (Table 5) (13), 13-*cis*-ß-carotene being formed in largest amount, followed by 13-*cis*-lutein and 15-*cis*-α-carotene. Canning (121°C, 30 min) resulted in the greatest loss of carotenoids, followed by high-temperature short-time heating at 120°C for 30 sec, 110°C for 30 sec, acidification plus 105°C heating for 25 sec and acidification. The carrot juice color turned from orange to yellow with intensive treatment.

Carrot juice was acidified, pasteurized and then subjected to lighted and dark storage at 4, 25 and 35°C for three months (14). Reduction of lutein, α-carotene and ß-carotene concentrations increased with increasing storage temperature and was also greater under illumination than under dark storage. The formation of 13-*cis*-isomers appeared to be favored under lighted storage and the 9-*cis*-isomers in the dark.

TABLE 4

Cis-trans ß-carotene isomer concentrations (µg/g dry wt)* in raw and processed sweet potatoes

Treatment	13-*Cis*-	All-*Trans*	9-*Cis*
Raw product	22	418	-
Strips (2-min blanch 100°C)	39	460	-
Strips (10-min blanch 100°C)	70	388	-
Puree (lye peeled, Fitzmill comminutor with 0.06" screen)	25	461	-
Steam injection (81°C to gelatinize starch, hold 30 min)	34	461	-
Steam injection (100°C to inactivate amylases)	37	419	
Canned (still retort, 90 min at 116°C)	57	323	11
Dehydrated (drum dried at 160°C at 25 rpm with contact time of 1.8-2 sec)	101	249	tr
Microwaved (full power for 7 min until internal temp. of 99°C)	56	284	tr
Baked (conventional oven at 191°C 80 min until internal temp. of 99°C)	69	232	tr

*Means of two replicate samples for each treatment. Tr - trace
Reference: Chandler and Schwartz (12).

TABLE 5

Cis-trans isomer concentration (µg/ml) changes of α-carotene, ß-carotene and lutein of carrot juice under various processing treatments

Carotenoid	Control	Acidified	I	II	III	IV
α-Carotene						
all-*trans*-	28a	26a	25a	15b	13c	11d
9-*cis*-	0.2a	0.2a	0.2a	0.4b	0.5c	0.5c
13-*cis*-	0.2a	0.3a	0.2a	0.6d	0.7e	0.5c
15-*cis*-	0.0a	0.0a	0.0a	1.5b	2.1d	1.3c
ß-Carotene						
all-*trans*-	62a	61a	60a	34b	33b	28c
9-*cis*-	1.1a	1.1a	1.2b	2.5c	3.1d	4.8e
13-*cis*-	3.4a	3.5a	4.5b	8.0c	11d	7.7c
15-*cis*-	1.1a	1.2a	1.5b	2.6c	3.3e	3.0d
13,15-di-*cis*-	1.3a	1.4a,b	1.4b	1.7c	1.9d	2.8e
Lutein						
all-*trans*-	6.0a	5.2b	4.6c	4.2c	3.2d	3.0e
9-*cis*-	0.4a	0.4a	0.5b	0.4a	0.6c	0.6c
13-*cis*-	0.6a	0.7a	0.8b	0.9c	1.5d	1.5d

Values in the same row bearing different letters are significantly different ($p < 0.05$). Carrot juice acidified to pH 4.0, heated at 105°C for 30 sec (I), juice (pH 6.1) heated at 110°C for 30 sec (II), juice (pH 6.1) heated at 120°C for 30 sec (III), juice (pH 6.1) heated at 121°C for 30 min (IV) for canning.
Reference: Chen et. al. (13).

Pesek and Warthesen (15) studied carotenoid photodegradation in vegetable juice containing mainly tomato and carrot juice, which had been exposed to 230 ft-c of light at 4°C. After four days of light exposure, only 25 percent of the initial α- and ß-carotene remained, while 75 percent of lycopene was still present. Structural differences were considered responsible for the difference in the degradation rates. Carotene loss was extensive after eight days. The control samples (held in darkness) showed no or negligible destruction of carotenoids.

To minimize hydrolytic rancidity in the oil, red palm fruits are sterilized immediately after harvest to inactivate lipases. Though necessary, this treatment (128°C, 66 min) provokes substantial isomerization, as shown in Table 6 for oils from *Elais guineensis* and *E. oleifera* fruits (16).

TABLE 6

Carotenoid composition (µg/g) of palm fruit oils

	From fresh fruits/From sterilized fruits			
Carotenoid	*E. guineensis* Dura Dumpy	*E. guineensis* Psífera	*E. guineensis* Tenera	*E. oleifera*
Cis-phytofluene	28/-	15/-	8.9/-	25/-
13-*Cis*-α-carotene	4.8/87	0.5/5.5	4.5/64	-/144
All-*trans*-α-carotene	296/228	18/14	164/94	425/342
13-*Cis*-ß-carotene	12/200	8.2/63	13/129	61/352
All-*trans*-ß-carotene	576/255	202/88	363/229	1026/400
9-*Cis*-ß-carotene	12/179	1.2/55	1.7/53	-/241
ζ-carotene	13/21	1.1/3.3	10/5.0	-/-
Zeaxanthin	tr/tr	6.0/tr	tr/tr	tr/tr
ß-Cryptoxanthin	tr/tr	tr/tr	tr/tr	31/14
Poly-*cis*-lycopene	41/4.7	8.8/1.2	22/-	-/-
Mono-*cis*-lycopene	7.4/38	3.5/4.8	3.1/7.1	-/tr
All-*trans*-lycopene	17/22	0.7/4.5	7.3/9.3	tr/tr
Unidentified	113/125	17/16	63/40	8.4/14
Total	1121/1160	283/255	660/631	1577/1506

All fruits were collected from the same experimental station. For each type of fruit, the oil sample was prepared from three bunches of fruits, part of which was sterilized before oil extraction.
Reference: Trujillo-Quijano et. al. (16).

The traditional method of palm oil production retained more ß-carotene (80 percent) than a mechanized process (23 percent) (17). The explanation was that the palm fruits processed in the traditional manner were not exposed to very high temperatures. When palm oil was heated to 160 to 200°C, the destruction rate of ß-carotene doubled for every 20°C rise in temperature.

Since pigment destruction was evident in commercial juices (18), pasteurization of guava juice was simulated in a pilot plant, the immersion of the bottled juice in boiling water being purposely extended to 30 min, double the usual pasteurization time needed for this type of product (19). Thermal treatment also involved blanching of the fruits for 5 min and heating of the juice up to a temperature of 87°C in a steam-jacketed kettle before hot filling. Pigment alteration was much less drastic than that noted in commercial juices at that time. *Cis*-lycopene increased five-fold on processing, but reduction of *trans*-lycopene was slight and statistically insignificant (Table 7). Both isomers decreased on storage (Table 8). The small amount of ß-carotene was retained during processing and storage.

Aside from geometrical isomerization, epoxidation and transformation of the 5,6-epoxide group to the 5,8-furanoid oxide are common alterations of carotenoids during heat treatment (Figure 2).

FIGURE 2
Formation of epoxy carotenoids from ß-carotene and transformation of violaxanthin during processing and storage of foods

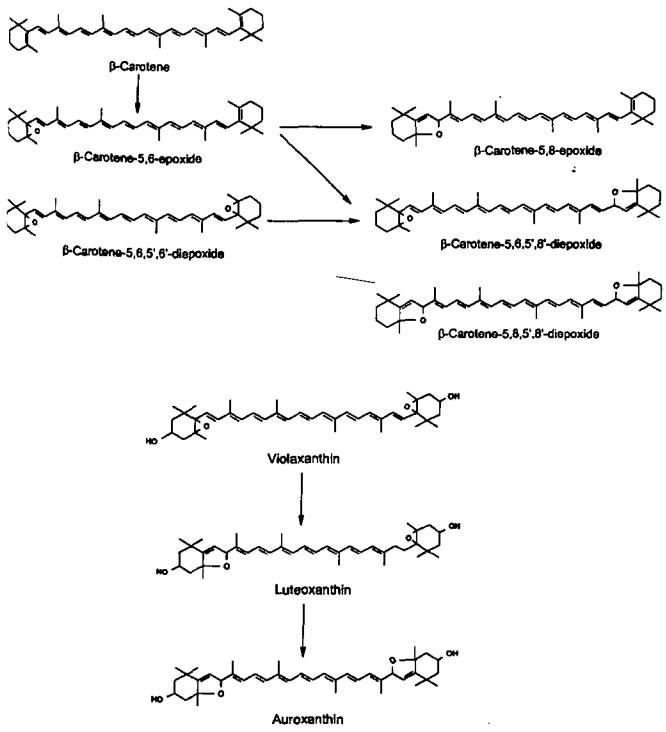


TABLE 7
Effect of processing on the carotenoids (µg/g)* of guava cultivar IAC-4 juice

Carotenoid	Fresh juice	Processed juice
ß-Carotene	2.7a	2.7a
ζ-carotene	tra	0.2b
γ-carotene	tra	0.1a
Zeinoxanthin	0.8a	0.8a
Cis-lycopene	1.2a	7.8b
Lycopene	31a	27a
Trihydroxy-ß-carotene-5,8-epoxide	2.9a	0.3b
ß-carotene-5,6,5',6'-diepoxide	tra	tra
Total	39a	39a

*Means of two determinations. Values in the same row bearing different letters are significantly different ($p < 0.05$). Tr-trace.
Reference: Padula and Rodriguez-Amaya (19).

TABLE 8
Carotenoid (µg/g)* changes during ambient storage of processed guava cultivar IAC-4 juice

Carotenoid	Storage time (month)				
	0	1	4	7	10
ß-Carotene	2.7a	2.4a	2.5a	2.5a	2.5a
ζ-Carotene	0.2a	0.2a	0.3a	0.2a	0.3a
γ-Carotene	0.1ab	nda	0.3b	0.3b	0.2ab
Zeinoxanthin	0.8a	1.0a	0.6a	1.3a	1.2a
Cis-lycopene	7.8a	7.9a	6.6a	3.5b	2.9b
Lycopene	27a	25ab	23ab	22ab	20b
Trihydroxy-ß-carotene-5,8-epoxide	0.3a	2.9c	0.6a	1.8b	1.6b
ß-carotene-5,6,5',6'-diepoxide	tra	nda	0.2a	0.1a	nda
Zeinoxanthin-5,8-epoxide	nda	nda	tra	tra	tra
Total	39ab	40a	34abc	32bc	29c

*Means of two determinations. Values in the same row bearing different letters are significantly different. Tr - trace, nd - not detected.
Reference: Padula and Rodriguez-Amaya (19).

The carotenoid composition was practically maintained on processing mango (cultivar Tommy Atkins) slices (Table 9) (20). Thermal treatment consisted of immersion of the sealed cans in boiling water for 20 min. The only significant change was the increase in luteoxanthin, compatible with the conversion of 5,6- to 5,8-epoxide. More evident changes occurred on processing mango (cultivar Golden) purée, which involved heating of the purée in an open, steam-jacketed kettle to 80°C for 10 min and immersion of the hot-filled and sealed cans or bottles in boiling water for 10 min. ß-carotene decreased 13%. Auroxanthin, not found in the fresh fruit, appeared while violaxanthin and luteoxanthin decreased, again reflecting the transformation of 5,6- to 5,8-epoxide. During storage of mango slices in lacquered (epoxy) or plain tin-plate cans, no appreciable loss of ß-carotene was observed during 10 months (Table 9) at ambient conditions. Between the tenth and

fourteenth month, about 50% reduction of ß-carotene occurred. The degradation continued, resulting in a total loss of 84% after 24 months. Violaxanthin tended to decrease and auroxanthin to increase during storage. ß-Carotene showed a greater tendency to degrade in bottled mango purée (18% loss after 10 months) than in the canned purée. As in mango slices, however, both bottled and canned purée suffered 50% loss of ß-carotene after the tenth month, and total loss of 83% after 24 months. Violaxanthin and luteoxanthin tended to decrease while auroxanthin maintained a comparatively high level throughout storage.

TABLE 9
Changes in ß-carotene (µg/g)* on storage of mango (cultivar Tommy Atkins) slices and mango (cultivar Golden) purée

Product/Packaging	Storage time (month)						
	0	1	3	7	10	14	24
Mango slices							
- in lacquered epoxy cans	14a	14a	14a	13a	14a	7.6b	2.3c
- in plain tin-plate cans	14a	12a	12a	12a	12a	6.7b	2.3c
Mango purée							
- in lacquered epoxy cans	16ab	15b	16a	15b	15b	8.0c	2.8d
- bottled	16a	15ab	15ab	12c	13bc	7.5d	2.6e

*Means of two determinations. Values in the same row bearing different letters are significantly different ($p \leq 0.05$).
Reference: Godoy and Rodriguez-Amaya (21).

In a recent paper (21), violaxanthin was found to be actually the major carotenoid of two mango cultivars. Notoriously unstable, violaxanthin can be easily lost during analysis, probably leading researchers to underestimate its concentration in earlier papers. In commercially processed mango juice (three brands), violaxanthin was not detected while auroxanthin was found in appreciable amount and ß-carotene became the principal carotenoid.

In papaya (cultivar Solo) purée, processed in the same manner as the mango purée, no significant loss of ß-carotene, ζ-carotene and γ-carotene occurred during processing (Table 9) (22). There was a small significant decrease in ß-cryptoxanthin. *Cis*-lycopene increased seven-fold, but the slight decrease in *trans*-lycopene was statistically insignificant. Cryptoflavin, an epoxy derivative of cryptoxanthin, appeared on processing. During 14 months of ambient storage, ß-carotene, *trans*-lycopene, and *cis*-lycopene did not change significantly, although the first two carotenoids showed a slight downward trend (Table 10). ß-cryptoxanthin did not change significantly during the first 10 months, but showed a small significant decrease after the fourteenth month of storage. Auroxanthin and flavoxanthin were formed during storage.

TABLE 10
Effect of processing on the carotenoid composition (µg/g)* of papaya (cultivar Solo) purée

Carotenoid	Fresh	Processed
ß-Carotene	2.6a	2.3a
ζ-Carotene	1.5a	1.3a
γ-Carotene	0.2a	0.2a
ß-Cryptoxanthin	7.4a	5.5b
Trans-lycopene	28a	23a
Cis-lycopene	0.2a	1.5b
Cryptoflavin	nd	0.2
Total	40a	34a

*Means of two determinations. Values in the same row bearing different letters are statistically different ($p < 0.05$). Nd - not detected.
Reference: Godoy and Rodriguez-Amaya (22).

In industrial dehydration (hot-air drying at 65°C) and lyophilization (freezing at -30°C and lyophilization at -10°C) of spinach previously immersed in salt and bicarbonate solutions, only a 12 percent loss of ß-carotene occurred and lutein, violaxanthin and zeaxanthin did not change significantly in both drying methods (23). These losses are small, considering the drastic processing treatment involved in dehydration and the greater exposure to oxygen. Sixty-seven % of all-*trans*-ß-carotene was retained after freeze-drying Italian spinach and 57 to 62 percent after solar-drying Italian spinach, spring cabbage and cowpea leaves (24).

TABLE 11
Changes in carotenoid composition (µg/g)* of bottled papaya purée during ambient storage

Carotenoid	Storage time (month)					
	0	1	3	6	10	14
ß-Carotene	2.3a	2.7a	1.9a	2.1a	1.9a	1.8a
ζ-Carotene	1.3a	0.9a	1.2a	0.9a	1.1a	1.3a
γ-Carotene	0.1a	0.2a	0.2a	0.2a	0.1a	0.3a
ß-Cryptoxanthin	5.5a	5.1a	4.8a	4.9a	5.1a	4.0b
Trans-lycopene	23a	22a	22a	22a	20a	21a
Cis-lycopene	1.5a	1.9a	1.6a	1.4a	2.1a	1.9a
Cryptoflavin	0.2a	0.6a	0.3a	0.3a	0.2a	0.3a
Flavoxanthin	nd	0.3a	0.2a	0.2a	0.3a	0.6b
Auroxanthin	nd	nd	1.0a	0.7b	1.1a	0.9a
Total	34a	34a	33a	32a	35a	32a

*Means of two determinations (three bottles were mixed for each determination). Values in the same row bearing different letters are significantly different ($p < 0.05$).
Reference: Godoy and Rodriguez-Amaya (22).

Among the various forms of processed foods, dried or dehydrated products are considered more likely to undergo

carotenoid degradation during storage because of the increase in surface area and porosity, the latter being associated with lyophilized (freeze-dried) foods.

Changes in carotenoid composition during blanching and storage of frozen and freeze-dried winter squash were investigated by Kon and Shimba (25). There was no loss of carotenoids during blanching. Lutein decreased slightly but ß-carotene was stable during freezing. Under frozen storage, lutein was stable while ß-carotene decreased 32% after three months. No loss of carotenoids was observed on freeze-drying. During storage of freeze-dried squash at 30°C, loss of ß-carotene reached 15, 20 and 53% after one, two and three months, respectively. However, after 3 months of storage at 3°C, reduction of ß-carotene was only 10%.

Processing of paprika

The influence of industrial processing of paprika on carotenoid composition was investigated by Minguez-Mosquera et al. in a series of studies. The drying and milling stages did not affect all of the pigments equally (26). The yellow pigments, particularly ß-carotene, were the most unstable; the red pigments (capsanthin and capsorubin) were highly stable. In drying the pepper variety Bola at 35°C, a period of continued carotenoid biosynthesis occurred (27), which was strongly favored by light. At the final stages of drying, light had a strong degradative effect. It was suggested that in order to obtain dry peppers for paprika with a 20 to 40 percent increase in carotenoid concentration, the drying process should consist of a first phase of illumination and a second phase of darkness. Two industrial drying processes were compared: slow drying with wood combustion and fast drying using hot air (28). The concentration of some pigments increased in Bola peppers dried with wood combustion, which was interpreted as a reflection of biosynthesis. During fast drying, degradative losses were evident.

Carotenoids in two varieties of peppers, Bola and Agridulce, behaved differently during drying (29). Capsanthin and capsorubin increased in the Bola variety and decreased in the Agridulce variety. In contrast, ß-cryptoxanthin and zeaxanthin went down in the Bola variety but increased slightly in the Agridulce pepper. The ß-carotene level was reduced in both varieties. All the carotenoids quantified decreased during milling. The Agridulce variety, which had higher carotenoid content, was found to be more suitable for paprika production, giving a final product with a more intense color and higher provitamin A content.

During slow industrial drying (30 to 35°C) of the Agridulce variety, three phases were discerned (30). In the first phase, there was a decrease of the carotenoid concentrations of the fruits. The second phase indicated an increase in carotenoid levels, although the previous losses were not compensated. In the third phase, degration prevailed. This pattern was observed under illumination and in darkness.

Fermentation

While other carotenoids underwent transformations, ß-carotene and lutein in olives resisted fermentation and the curing process (210 or 89 days) (31,32). The xanthophylls with 5,6-epoxide groups (violaxanthin and neoxanthin) were converted into their corresponding 5,8-furanoid derivatives (auroxanthin and neochrome), such transformations fitting first-order kinetics with respect to pigment concentration. In mustard, ß-carotene and lutein were reportedly reduced to one-third of their original contents after 50 days of curing (33).

Influencing factors and mechanism of carotenoid degradation

The factors that influence carotenoid degradation were discussed in detail in a previous paper (34). In model systems, carotenoid decomposition was shown to depend on carotenoid structure, nature of the system, available oxygen, exposure to light, water content or activity, temperature, atmosphere, presence of antioxidants, prooxidants, free radical initiators and inhibitors, sulfites. As shown in the previous sections, the situation is more complex in foods, considering the complicated interplay of the factors mentioned above, along with the varied nature and composition of foods, processing treatment, packaging and storage conditions, activity of lipoxygenase and other enzymes, and coupled oxidation with lipids. Nevertheless, application of available information makes this degradative process controllable.

In contrast to the wealth of information on lipid oxidation, present-day knowledge of carotenoid degradation is fragmentary. It is often accompanied by isomerization; both *cis*- and *trans*-isomers are subject to oxidation (Figure 3). It is generally accepted that the initial stage of oxidation involves epoxidation (Figure 3) and formation of apocarotenals (Figure 4). Recent work (35,36) show that hydroxylation is also involved. Presumably, subsequent fragmentations result in compounds of low molecular masses, which contribute to the desirable flavor of wine and tea but can be responsible for the off-flavor of dehydrated carrot. Full structural elucidation of the intermediate and final products of this process, as well as delineation of the mechanisms for their formation, are urgently needed.

Influence of processing on bioavailability

For a long time the main concern about processing in relation to carotenoids has been preventing losses. In recent years, attention has shifted to the effect of processing on the bioavailability of carotenoids.

Carotenoids in nature may occur as crystals, dissolved in oil droplets or complexed with protein, and protected by the cellular structure. This natural protection appears to limit its bioavailability. Processing denatures proteins and breaks down the cell walls, making the release of carotenoids from the food matrix easier.

FIGURE 3
Possible scheme for carotenoid degradation

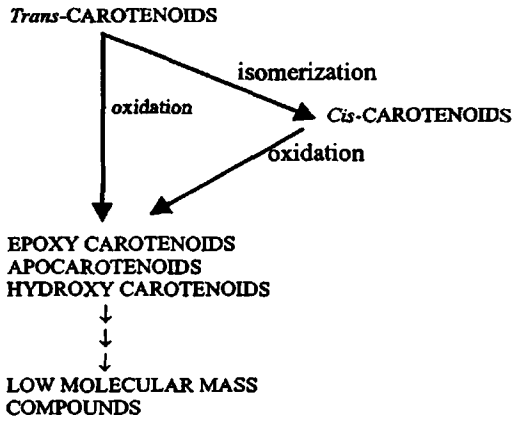
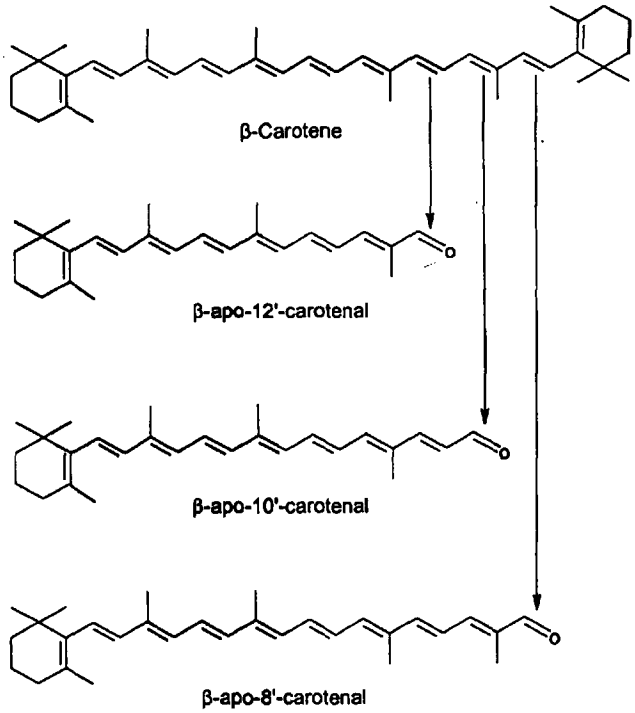


FIGURE 4
Formation of apocarotenals from ß-carotene



The carotenoid concentrations in the chylomicrons of five subjects given a single dose of fresh tomato or tomato paste were determined (36). The chylomicron carotenoid level is considered to be a more appropriate tool for studying absorption kinetics than plasma concentrations. Ingestion of tomato paste resulted in 2.5-fold higher total and all-*trans*-lycopene peak concentrations and 3.5-fold higher total area under the curve than ingestion of fresh tomato. Thus, the bioavailability of lycopene in humans appears greater from tomato paste than from fresh tomatoes.

In an earlier study based on plasma concentration, the uptake of lycopene was found to be greater from heat-processed (cooked in an oil medium) than from unprocessed tomato juice (37). Ingestion of cooked tomato juice resulted in a two- or three-fold increase in lycopene serum concentrations one day after ingestion. An equivalent consumption of unprocessed tomato juice caused no rise in plasma concentrations.

This topic is being investigated by several research groups. It is hoped that processing under appropriate conditions can serve as a means of enhancing bioavailability, at the same time retaining the carotenoid content of foods.

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REFERENCES

1. Rodriguez-Amaya DB. Carotenoids and food preparation: The retention of provitamin A carotenoids in prepared, processed, and stored foods. Arlington: Opportunities for Micronutrient Intervention (OMNI), 1997.
2. Cavalcante ML, Rodriguez-Amaya DB. Alteration of the carotenoid composition during manufacture and storage of frozen *Eugenia uniflora* fruit. Proceedings of the 9th World Congress of Food Science and Technology; 1995 July 30 - Aug 4; Budapest: International Union of Food Science and Technology, 1995.
3. Gross J. Pigments in fruits. London: Academic Press, 1987.
4. Rodriguez-Amaya DB. Nature and distribution of carotenoids in foods. In: Charalambous G, editor. Shelf-life studies of foods and beverages. Chemical, biological, physical and nutritional aspects. Amsterdam: Elsevier Science Publishers, 1993:547-89.
5. Oruña-Concha MJ, González-Castro MJ, López-Hernández J, Simal-Lazano J. Effects of freezing on the pigment content in green beans and padrón peppers. Z Lebensm Unters Forsch A 1997; 205:148-52.
6. Wu Y, Perry AK, Klein BP. Vitamin C and ß-carotene in fresh and frozen green beans and broccoli in a simulated system. J Food Qual 1992;15:87-96.
7. Cano MP, de Ancos B, Lobo G, Monreal M. Effects of freezing and canning of papaya slices on their carotenoid composition. Z Lebensm Unters Forsch 1996; 202:279-84.
8. Bjerkeng B, Johnsen G. Frozen storage quality of rainbow trout (*Oncorhynchus mykiss*) as affected by oxygen, illumination, and fillet pigment. J Food Sci 1995; 60:284-8.
9. Lessin WJ, Catigani GL, Schwartz SJ. Quantification of *cis-trans* isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. J Agric Food Chem 1997; 45:3728-32.
10. Panalaks T, Murray TK. The effect of processing on the content of carotene isomers in vegetables and peaches. Can Inst Food Sci Technol J 1970; 3:145-51.

11. Sweeney JP, Marsh AC. Effect of processing on provitamin A in vegetables. J Am Diet Assoc 1971; 59:238-43.
12. Chandler LA, Schwartz SJ. Isomerization and losses of *trans*-ß-carotene in sweet potatoes as affected by processing treatments. J Agric Food Chem 1988; 36:129-33.
13. Chen BH, Peng HY, Chen HE. Changes of carotenoids, color, and vitamin A contents during processing of carrot juice. J. Agric. Food Chem 1995; 43: 1912-8.
14. Chen HE, Peng HY, Chen BH. Stability of carotenoids and vitamin A during storage of carrot juice. Food Chem 1996; 57:497-503.
15. Pesek CA, Warthesen JJ. Photodegradation of carotenoids in a vegetable juice system J Food Sci 1987;52:744-6.
16. Trujillo-Quijano JA, Rodriguez-Amaya DB, Esteves W, Plonis GF. Carotenoid composition and vitamin A values of oils from four Brazilian palm fruits. Fat Sci Technol 1990;92: 222-6.
17. Jideani VAE. Carotene retention in palm oil by mechanised and traditional processes. J Food Sci Technol 1992;29: 68-9.
18. Padula M, Rodriguez-Amaya DB, Moraes MAC. Comparison of the carotenoid composition and general properties of the processed juice of guava cultivar IAC-4 and commercial juices. Cienc Tecnol Aliment 1983; 3:109-16.
19. Padula M, Rodriguez-Amaya DB. Changes in individual carotenoids and vitamin C on processing and storage of guava juice. Acta Alimentaria 1987;16:209-16.
20. Godoy HT, Rodriguez-Amaya DB. Changes in individual carotenoids on processing and storage of mango (*Mangifera indica*) slices and purée. Int J Food Sci Technol. 1987;22: 451-60.
21. Mercadante AZ, Rodriguez-Amaya DB. Effects of ripening, cultivar differences, and processing on the carotenoid composition of mango. J Agric Food Chem 1998; 46:128-30.
22. Godoy HT, Rodriguez-Amaya DB. Comportamento dos carotenóides de purê de mamão (*Carica papaya*) sob processamento e estocagem. Cienc Tecnol Aliment 1991;11:210-20.
23. Ramos DMR, Rodriguez-Amaya DB. Avaliação das perdas de carotenóides e valor de vitamina A durante desidratação e liofilização industrial de espinafre. Arq Biol Tecnol 1993;36:83-94.
24. Nyambaka H, Ryley J. An isocratic reserved-phase HPLC separation of the stereoisomers of the provitamin A carotenoids (α- and ß-carotene) in dark green vegetables. Food Chem 1996; 55:63-72.
25. Kon M, Shimba R. Changes in carotenoid composition during preparation and storage of frozen and freeze-dried squash. Nippon Shokuhin Kogyo Gakkaishi 1989; 36: 619-24.
26. Minguez-Mosquera MI, Jarén-Galán M, Garrido-Fernández J. Effect of processing of paprika on the main carotenes and esterified xanthophylls present in the fresh fruit. J Agric Food Chem 1993;141: 2120-4.
27. Minguez-Mosquera MI, Jarén-Galán M, Garrido-Fernández J. Competition between the processes of biosynthesis and degradation of carotenoids during the drying of peppers. J Agric Food Chem 1994; 42:645-8.
28. Minguez-Mosquera MI, Jarén-Galán M, Garrido-Fernández J. Influence of the industrial drying processes of pepper fruits (*Capsicum annuum* cv. Bola) for paprika on the carotenoid content. J Agric Food Chem 1994; 42:1190-3.
29. Minguez-Mosquera MI, Homero-Méndez D. Comparative study of the effect of paprika processing on the carotenoids in peppers (*Capsicum annuum*) of the Bola and Agridulce varieties. J Agric Food Chem 1994; 42:38-44.
30. Minguez-Mosquera MI, Jarén-Galán M, Garrido-Fernández J. Carotenoid metabolism during the slow drying of pepper fruits of the Agridulce variety. J. Agric Food Chem 1994; 42: 2260-4.
31. Minguez-Mosquera MI, Garrido-Fernández J, Gondul-Rojas B. Pigment changes in olives during fermentation and brine storage. J. Agric Food Chem 1989; 37:8-11.
32. Minguez-Mosquera MI, Gondul-Rojas B. Mechanism and kinetics of carotenoid degradation during the processing of green table olives. J Agric Food Chem 1994; 42:1551-4.
33. Fan JJ, Lee HC, Yen GC. Quantification and identification of carotenoids and chlorophylls in raw and pickled mustard. J Chinese Agric Chem Soc 1993;31:765-75.
34. Rodriguez-Amaya DB. Stability of carotenoids during the storage of foods. In: Charalambous G, editor. Shelf-life studies of foods and beverages. Chemical, biological, physical and nutritional aspects. Amsterdam: Elsevier Science Publishers, 1993: 591-628.
35. Marty C, Berset C. Degradation produts of *trans*-ß-carotene produced during extrusion cooking. J Food Sci 1988; 53:1880-6.
36. Padula M, Rodriguez-Amaya DB. Degradação de ß-caroteno em sistema modelo à temperatura ambiente: Formação de compostos não-voláteis e voláteis. Proceedings of the Congresso Latino-Americano de Carotenóides em Alimentos; 1998 Sept 14-17; Campinas, Brasil.
37. Gärtner C, Stahl W, Sies H. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. Am J Clin Nutr 1997;66: 116-22.
38. Stahl W, Sies H. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice. J Nutr 1992; 122:2161-6.

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Processing characteristics and stability of chemically synthesized carotenoids in food systems

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SUMMARY. Product development is driven by consumer demand for variety in food. Increasing health awareness and a corresponding lifestyle are the main factors that influence the development of new food products. The pure nutritional aspect of food is becoming less and less important, leaving enjoyment and health as the major motivators of consumer preference. Research & Development and Technical Service departments for carotenoid formulations have been taking up these challenges for a long time. They have developed carotenoid formulations with processing characteristics and stability to meet the customer's requirements. Carotenoids that are approved under food laws are used as colorants. ß-carotene is also important as provitamin A and as a physiologically active substance. Carotenoid formulations are available as dispersions, powders and emulsions. They must meet the requirements of the different food production processes. Ease of handling, and stability to light, air and heat are important technical features of our carotenoid formulations. Some of the main processing parameters and the stability of carotenoid formulations are discussed with reference to the beverage industry, margarine production and the dairy industry.
Key words: Synthetic carotenoids, stability, processing characteristics

RESUMEN. Características de procesamiento y estabilidad de los carotenoides químicamente sintetizados en sistemas alimenticios. El desarrollo de productos es impelido por la demanda del consumidor por variedad en alimentos. La creciente concientización sobre la salud y el estilo de vida correspondiente son los factores principales que influyen en el desarrollo de nuevos productos alimenticios. El aspecto nutricional puro de los alimentos está siendo cada vez menos importante, dejando el placer y la salud como los mayores motivadores de la preferencia del consumidor. Los Departamentos de Investigación y Desarrollo y Servicios Técnicos para formulaciones con carotenoides han tomados para sí estos desafíos por un largo tiempo. Ellos han desarrollado formulaciones de carotenoides con características de procesamiento y estabilidad que atienden las exigencias del cliente. Carotenoides aprobados por legislación son usados como colorantes. ß-Caroteno es también importante como provitamina A y como una sustancia fisiológicamente activa. Formulaciones de los carotenoides se encuentran disponibles como dispersiones, polvos y emulsiones. Ellos deben atender los requisitos de los diferentes procesos de producción de alimentos. Facilidad de manipulación, y estabilidad a la luz, aire y calor son importantes propiedades de nuestras formulaciones de carotenoides. Algunos de los principales parámetros de procesamiento y la estabilidad de formulaciones de carotenoides son discutidos en relación a la industria de bebida, producción de margarina y la industria lechera.
Palabras clave: Carotenoides sintéticos, estabilidad, características de procesamiento

INTRODUCTION

In recent years, consumer demand has developed more in the direction of products with refreshing properties that are associated with greater enjoyment and quality of life. Increasing health-awareness and a corresponding lifestyle are having a strong influence on the development of new food products. The pure nutritional function of food is receding ever further into the background. Enjoyment and health still remain the primary motives for consumer preference. And enjoyment also means variety. The subject of health is becoming increasingly important in a society with a strongly growing proportion of older persons. Consumers are open to new ideas, which they judge not only in terms of quality but also quite consciously in terms of the price/performance relationship.

R&D and Technical Service Departments for carotenoid formulations have always taken up these challenges. They have developed carotenoid formulations that give customers the processing characteristics and stability that customers require.

Carotenoid formulations as colorants

Food products can be coloured for the following reasons:

- to make up for colour lost in the production process, storage, packaging and distribution of the product
- to improve the brilliance of the existing colour of the food product
- to give food products a more attractive appearance and to

create an association with their flavour.

We tend to associate the colour of a food with its taste and flavour. For the consumer, the colour is also an indicator of the quality of the food product. An attractive colour increases the incentive to buy, leading to higher sales. The recipes and production processes for food products must aim to meet the interests and wishes of the consumer.

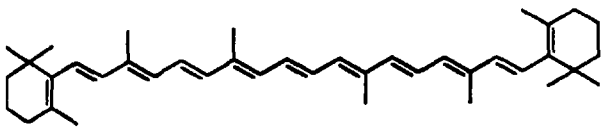
There are situations in which it is difficult to use colorants in foods:

- legal restrictions
- technical properties and stability of the colorant
- the unavailability of a suitable shade
- economic reasons; the colorant costs are too high compared with the overall costs.

Where do carotenoid formulations stand in these situations? Four synthetic carotenoids are currently used in food products:

- ß-carotene
- ß-apo-8-carotenal
- ß-carotenoic acid ethyl ester
- canthaxanthine

The main characteristics of ß-carotene are:



Solubility:	chloroform	very good
	vegetable oils	moderate
	water	insoluble

λ_{max} = 445 nm $A^{1\%}_{1\,cm}$ = 2500 Fp = 176 - 182 C

all-*trans*-ß-carotene $C_{40}H_{56}$

The use of canthaxanthine and apo-carotenal formulations is subject to different food laws in different countries. ß-Apo-8-carotenoic acid ethyl ester is used to colour (mark) butter fats.

There are practically no restrictions on the use of ß-carotene formulations. According to the EC Additives Directive, ß-carotene can be added to food products according to the *quantum satis* principle, i.e. the colorants can be used, according to GMP principles, in quantities that are no greater than are required to achieve an intended effect, though this must not deceive the consumer.

ß-Carotene formulations can be divided into

- ß-carotene dispersions
- ß-carotene powder formulations
- ß-carotene emulsions
- ß-carotene solubilizates (described in patents)

The ß-carotene dispersions consist of finely ground ß-carotene in vegetable oils. They are used to colour food products with a high oil content.

Margarine: In many countries, margarine is given its attractive colour with ß-carotene dispersions. The ß-carotene is present in dissolved form in the oil droplets and fat crystals of the margarine. The antioxidative components in the margarine make ß-carotene highly stable.

If the ß-carotene is not completely dissolved, the colour obtained is a paler red. The process conditions must be adjusted to ensure that the ß-carotene is always completely dissolved in the margarine.

Perhaps the reader has noticed, when shaving off a layer of margarine with a knife, that the margarine exposed has a much lighter shade. What has happened here is not that the ß-carotene in the margarine has decomposed, but that its concentration at the surface has increased.

This is because water evaporates from the margarine emulsion at the surface. If the difference is too conspicuous, and undesired, the processing conditions where the margarine is produced are probably not to blame; instead, the water vapour permeability of the packaging should be checked.

The replacement of vitamin A in margarine by provitamin A has been considered. However, ß-carotene has such a high coloration power that replacing vitamin A in margarine with provitamin A would give it too strong a colour. The solution here would be a non-colouring ß-carotene formulation. Unfortunately, there is as yet no such formulation for use in margarine.

It is also possible to use natural carotene and annatto to colour margarine. Annatto does not give the margarine the desired brilliance of colour and is not as stable as ß-carotene.

Economic reasons and variations in colour have prevented the success of natural carotene.

ß-Carotene formulations in milk products, e.g. yoghurt

Yoghurt preparations are given the shade that meets customers' wishes by adding ß-carotene powder formulations and ß-carotene emulsions. It is possible to improve the stability of ß-carotene in yoghurt by adding riboflavine.

One reason for the improvement in stability is that riboflavine absorbs light at 450 nm, which is practically the same as the corresponding wavelength for ß-carotene, 445 nm.

The proximity of the wavelength of maximum absorption (450nm) for ß-carotene and riboflavine can lead to problems in the quantitative analysis of multivitamin products.

Yoghurt often contains a fruit preparation. ß-Carotene powder can be added to this fruit preparation. Incorporating 10% powder formulations into fruit preparations can present a technological challenge.

10% powders contain amorphous ß-carotene, which is one reason why they give a much redder shade than the 1% powder

containing ß-carotene dissolved in oil. ß-Carotene can be extracted from the 10% powder. The dissolved ß-carotene shifts the shade in the direction of orange-yellow. By carefully controlling the production conditions, it is possible to adjust the shade in this way.

Pasta in Asia

In some countries, it is permitted to colour pasta products with ß-carotene. Instant noodles are given their attractive colour by adding approximately 4 ppm of ß-carotene.

Carotenoids for colouring beverages

Some segments of the food market are showing symptoms of saturation and increased competition - the driving force for the development of new products. Particularly in the beverage market there has been a large number of new products in recent years.

Yesterday's niches are becoming today's market sectors.

The number of different beverages is becoming ever larger. Their appearance and colour are the first and most important impression that affects the purchasing behaviour of the consumer. The beverage can be clear or turbid; it can have a wide range of different shades of colour between yellow and red-orange.

Carotenoid formulations are very important as colorants for beverages.

To meet the wide range of different processing conditions as well as the demands of the food industry, carotenoid formulations must be developed to meet the following challenges:

- color strength of the formulation
- consistency of shade from batch to batch
- good flow properties
- low dust formation
- good wettability
- good dispersibility in water
- narrowest possible particle-size distribution
- good chemical stability of the ß-carotene in the dry powder
- good chemical and physical stability in foods
  - light stability
  - opalescence, if required
  - lowest possible sensitivity to Ca
  - no changes in the pH range of the isoelectric point
  - no tendency to creaming
- kosher quality

The following processing parameters must be observed when handling ß-carotene powder formulations and emulsions:

- proper preparation of the stock dispersion
- proper preparation of a stock emulsion
- homogenization parameters
- oxidation protection, enhancement of light stability

The following processing conditions should be maintained to obtain squashes and non-carbonated beverages with a shelf life of 6-12 months:

1. Use the recommended colorant concentration
2. Keep the copper concentration below 0.5 ppm
3. Keep the iron concentration below 0.3 ppm
4. Add ascorbic acid and the colorant as late as possible in the process
5. Use a relatively low ullage and a relatively high filling temperature

Natural colorants like anthocyanins are often heat-sensitive, react to changes in pH value, lack colour strength, can affect the flavour of beverages, and their shade can vary.

ß-Carotene formulations have a particularly high colour strength, consistency of shade, are free flowing and dispersible in cold water, as well as being chemically and physically stable in the beverage.

The ß-carotene powder and emulsion formulations have no tendency to cream. However, aroma oils or other components in the beverage can separate out and form a ring on top of the beverage at the beverage/glass/air boundary.

ß-Carotene is soluble in oil and colours the otherwise colourless ring. Consumers who spot this frequently believe the beverage is of inferior quality.

The separation phenomenon can be minimized by careful attention to the processing parameters, i.e. the recipe for the beverage, the point at which the ß-carotene is added and the homogenization parameters.

Vitamin C or d,l-alpha-tocopherol are added to stabilize the ß-carotene in the beverage.

ß-Carotene powder formulations demonstrate good stability even in alcoholic beverages (4% by volume).

There are on the market carotene formulations of different colour intensity in the yellow-orange and orange-red to red ranges. Depending on the beverage manufacturer's processing parameters, it is therefore possible to select the most suitable ß-carotene formulation with the greatest colour yield in the desired colour range.

One question that is frequently asked about processing is: Is it possible to use more of the 1% formulation to achieve the same shade of colour as the 10% formulation?

It would be logical to expect this, but it is not so. The reason is that the products use different carrier materials and the ß-carotene is present in different physical forms. A higher concentration of the 1% formulation gives a deeper yellow-orange shade.

The colour of a beverage strongly influences the purchasing behaviour of the consumer. It is not possible to colour all beverages. Thus, for instance, it is not permitted to colour fruit juices. The riboflavine contained in multivitamin fruit juices, and particularly the ß-carotene formulation have a major effect on the colour and appearance of the multivitamin fruit

juice. With the same concentration of provitamin A in a fruit juice, the differences in shade can be seen clearly.

Thus carotenoids, as provitamins or physiologically active ingredients, can significantly affect the shade of colour of a fruit juice. Suitable carotenoid formulations can therefore be used to better fulfil the wishes of the consumer.

Health food products with ß-carotene

Health food products are continuing to enjoy increasing popularity. Many soft gelatin capsule products and effervescent tablets comply with the food laws in Germany.

Differences in the particle-size distribution of the ß-carotene in the dispersions used in non-coloured soft gelatin capsules can give different colour impressions. The finer the ß-carotene is ground, the lighter it appears in the capsule. The conditions under which ß-carotene crystals are milled can play a decisive role here.

The demand for ß-carotene-containing tablets of food quality is growing. Carotene formulations must withstand pressures of several tonnes during tablet compression. The matrix must not disintegrate and no carotene must be expressed, as it would be oxidized by the air with a measurable loss.

ß-Carotene formulations were developed with tabletting conditions in mind.

Effervescent tablets are particularly popular in Germany. The compression conditions for effervescent tablets are much the same as those for any other tablet. However, it must not only withstand the high compression forces, it must also be dispersed readily in cold water. Effervescent tablets containing 10% ß-carotene formulations lead to a reddish or orange colour in the beverage.

Liquid multivitamin products are also available on the market as syrups; a ß-carotene emulsion can be used to fortify these syrups. It is possible to produce turbid and clear liquid formulations. This is possible only to a limited extent with the powder formulations.

Innovative carotene formulations

ß-Carotene formulations with a soya protein matrix are completely new to the market. There is an increasing demand for food products that are free of animal proteins, e.g. for vegetarians. According to our practical experience to date, the production parameters of food products made with ß-carotene formulations with soya do not differ significantly from those made with gelatin-based formulations. There are also no obvous differences in the stability of the ß-carotene.

To summarize:

Some of the factors that must be taken into account in processing chemically synthesized carotenoids in food products have been mentioned:

Margarine production
- proper dissolution of carotenoids
- correct emulsifying properties
- suitable packaging

Milk products
- auxiliaries such as riboflavine to enhance the light stability of the carotenoids
- correct processing parameters for yoghurts to avoid colour shifts

Properties of carotene formulations for production of
- plain tablets
- effervescent tablets
- beverages

Processing of carotene in food is straightforward and stability is no problem when the right carotene formulations are used.

CONCLUSIONS

In conclusion, it can be said that, of all the carotenoids approved for use in food products, ß-carotene can be used in most foods in almost all countries. A number of examples was given to show that the processing properties of the carotene formulations must meet a wide range of different requirements. The stability of the carotenoid is also affected by the composition of the dispersion, powder or emulsion formulation. The final colour of the food product is affected both by the type of carotenoid formulation and by the processing parameters for the food product. Because of their color strength and versatility, carotenoid formulations are economical. We can look forward to new carotenoid formulations and their processing technology challenges.

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Chromatographic separation of carotenoids

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SUMMARY. The carotenoids are extremely reactive and consequently unstable due to their long system of conjugated double bonds. Several precautions, such as protection against light and oxygen, use of low temperature and antioxidants, analysis in the shortest possible time, should be taken during isolation and chromatography. The food samples, preferably fresh, are homogenized and immediately extracted with a suitable organic solvent. Saponification has been employed in order to hydrolyze the carotenoid esters, remove fatty material and destroy chlorophyll. This optional step facilitates subsequent carotenoid separation, identification and quantification. The separation of carotenoids is usually carried out by column chromatography, thin layer chromatography and high performance liquid chromatography, in analytical or preparative scale, on many stationary phases such as silica-gel, alumina, MgO, $Ca(OH)_2$ and reversed-phase material (C_{18} and C_{30}). The choice of the most suitable chromatographic method depends on the amount of sample, carotenoid composition, resolution, speed and purity required. Examples of carotenoid separation in different stationary phases will be shown and discussed.
Key words: Carotenoids, isolation , chromatography.

RESUMO. Separação cromatográfica de carotenóides. Devido ao longo sistema de duplas ligações conjugadas, os carotenóides são altamente reativos e consequentemente instáveis. Várias precauções, tais como condução da análise no menor tempo possível, exclusão de oxigênio, proteção contra luz, uso de baixa temperatura e de antioxidantes, devem então serem tomadas durante o isolamento e cromatografia. Os alimentos, preferencialmente *in natura*, são homogenizados, e imediatamente os carotenóides são extraídos com solventes orgânicos. A saponificação é empregada com o objetivo de hidrolisar os ésteres de carotenóides, promover retirada de lipídeos e destruição de clorofila. Esta etapa opcional facilita a posterior separação, identificação e quantificação dos carotenóides. Os carotenóides são separados por cromatografia em coluna, cromatografia em camada delgada ou cromatografia líquida de alta eficiência (HPLC), tanto em escala analítica como preparativa. Várias fases estacionárias podem ser empregados tais como alumina, sílica, $Ca(OH)_2$, MgO e fase reversa (C_{18} e C_{30}). Os principais fatores que devem ser considerados na escolha do método cromatográfico são: quantidade de amostra, composição de carotenóides, pureza, resolução e velocidade necessárias. Serão apresentados e discutidos exemplos de separação de carotenóides em diversas fases estacionárias.
Palavras chave: Carotenóides, isolamento, cromatografia.

INTRODUCTION

The characteristic system of conjugated double bonds in the carotenoid molecule, in which the π electrons are delocalized over the whole polyene chain, is responsible for the long and straight shape of the molecule, for color due to absorption of visible light and for chemical reactivity, resulting in unstable and easily destroyed compounds.

Due to this fact, the following precautions must be taken during work-up:

- use of inert atmosphere, replacing air by vacuum or inert gas (N_2 or Ar),
- avoidance of high temperature, lower than 35°C for evaporation of large amount of solvent in the rotary evaporator; alternatively, evaporation of small volumes directly under N_2 or Ar,
- storage at very low temperature,
- all operations carried out in diffuse light; equipment and glassware covered by black cloth or aluminium foil,
- avoidance of acid and alkali, strongly acidic reagents not being used in the laboratory where the carotenoids are handled,
- all operations carried out in the shortest possible time.

Pre-chromatographic steps

The most common problem during work-up is *cis-trans* isomerization in solution catalyzed by heat, light, acids and active surfaces (1). Therefore, the pure carotenoid or even the crude extract should never be stored in solution, and preferably kept dry under inert atmosphere.

The steps involved in the preparation of the carotenoid extract are briefly discussed below.

Sample. It is important to use fresh and undamaged food samples, since unwanted reactions catalyzed by enzymes and acids may occur after harvesting.

Tissue homogenization should be preferably done immediately before analysis or during extraction because mechanical desintegration can introduce air and destruction of the cell wall liberates enzymes, such as lipoxygenase, and acids causing degradation of carotenoids and epoxide-furanoxide rearrangement, respectively.

Extraction. The choice of the best solvent for extraction depends on the sample, its pre-treatment and the carotenoid composition. Water-miscible organic solvents, usually acetone, are generally employed for extraction of fresh foods. Dried or lyophilized samples can be extracted with water immiscible solvents, such as ethyl acetate or diethyl ether (2), or preferably re-hydrated in the case of quantitative extraction, as for squashes and pumpkins (3) and pasta (4). The extraction of samples containing carotenoid glycosides demands a more polar solvent and usually ethanol (5) or methanol or its combination with acetone (6) is employed.

The addition of $NaHCO_3$, $MgCO_3$ or $CaCO_3$ (0.1 g/g sample) and antioxidants is recommended to neutralize tissues containing acids and to avoid oxidation, respectively.

The extraction can be carried out in a blender or simply with the aid of a mortar and pestle.

For qualitative extraction, large amounts of food are often used and there is no concern about loss of material. If the final objective is, for example, the isolation of a carotene, it is very useful to extract the sample first with a very polar solvent (e.g. methanol) that would remove water and the xanthophylls partially, both of which are discarded. The carotenes are then extracted with a suitable less polar solvent.

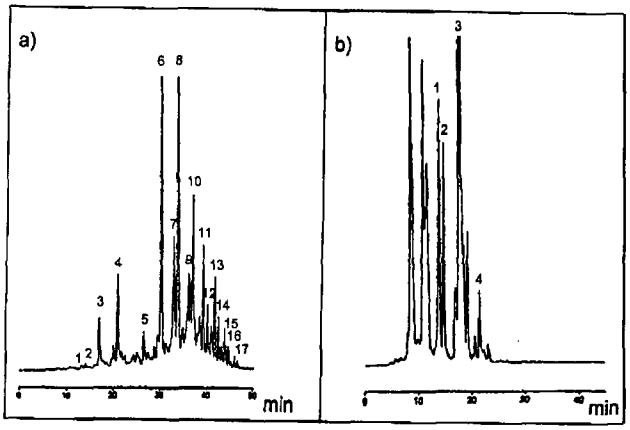
On the other hand, quantitative extraction requires complete and exhaustive extraction and no material can be lost. Usually three to four extractions are enough to remove the carotenoids completely from the sample.

Removal of water and solvents. The carotenoid extract obtained by extraction of the fresh sample with acetone contains large amounts of water which comes from the sample. Therefore, in order to remove water and acetone, the carotenoids are transferred to petroleum ether or diethyl ether by adding small portions of the acetone extract and large amount of water in a separatory funnel. The remaining traces of water can be removed either by addition of anhydrous Na_2SO_4 or drops of ethanol (by formation of an azeotropic mixture).

Saponification. Alkaline hydrolysis (saponification) has been used to remove contaminating lipids from fat-rich samples (e.g. palm oil), destroy chlorophyll (e.g. green vegetables) and hydrolyze carotenoid esters.

Xanthophylls esterified with a mixture of different fatty acids are typically found in fruits, and the use of saponification allows an easier chromatographic separation, identification and quantitation. This fact is exemplified with a carotenoid extract from tangerine, shown in Figure 1, where among the carotenoid esters, the unsaponificable extract contains free ß-cryptoxanthin and six esters of this major carotenoid. As expected, on saponification, the chromatogram turns to be much simpler and ß-cryptoxanthin becomes the major carotenoid (7).

FIGURE 1
HPLC chromatogram of a) unsaponified and b) saponified tangerine extract



Identification of numbered peaks: 1-lutein, 2-zeaxanthin, 3-ß-cryptoxanthin, 4-ß-carotene, 5 to 10- esters of ß-cryptoxanthin, 11 to 17-esters of di- and poly-hydroxy xanthophylls. Conditions: C_{18} Suplex pKb 100 (5µm, 4.6 x 250 mm) column and MeOH/acetonitrile/CH_2Cl_2/hexane (10:85:2.5:2.5) for 5 min, going to 10:45:22.5:22.5 from 5 to 40 min at 0.7 ml/min. Reference: Wingerath et al. (7).

Although saponification was found to be unnecessary for separation and quantification of carotenoids from leafy vegetables by column chromatography (CC) (8, 9), saponification is most of the time employed to clean the extract when subsequent identification is done by mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy.

A general procedure that has been employed in our laboratory is the addition of an equal amount of methanolic 10% KOH to the hexanic carotenoid extract. This solution can be bubbled with N_2 or Ar and allowed to stand overnight at room temperature. Subsequently, the mixture is washed with water in a separatory funnel until free of alkali.

Carotenoids with 3-hydroxy-4-keto group, as astaxanthin, which is widespread in marine animals, microorganisms and algae, undergo oxidation in the presence of alkali and air. For such samples, saponification is not recommended or must be carried out under anaerobic conditions. For this purpose, a special apparatus and procedure were developed (10).

Aldol condensation is another undesirable reaction that can produce artifacts such as conjugated methyl ketones. Carotenals undergo aldol condensation during saponification in the presence of acetone that remains from the extraction

step. In fact, citranaxanthin and reticulaxanthin, reported as natural carotenoids from citrus, are probably aldol condensation products formed from ß-apo-8'-carotenal and ß-citraurin, respectively (11). In such samples the extraction can be performed with methanol and ethyl acetate.

It is recommended to verify if structural changes occur during saponification. Since the hydroxyl groups have no influence on the chromophore, the wavelength of the maximum absorption, shape and intensity of the UV-visible spectrum would be identical for the unsaponified and saponified samples.

The use of plastic material, filter paper and blender during the steps described above should be avoided, in order to prevent contamination, if the isolated carotenoid would be analyzed by direct insertion in the mass spectrometer.

Chromatography

The separation of carotenoids can be carried out by CC, thin layer chromatography (TLC), high performance liquid chromatography (HPLC) or a combination thereof. Since carotenoids are labile to high temperature, gas chromatography is not employed for separation.

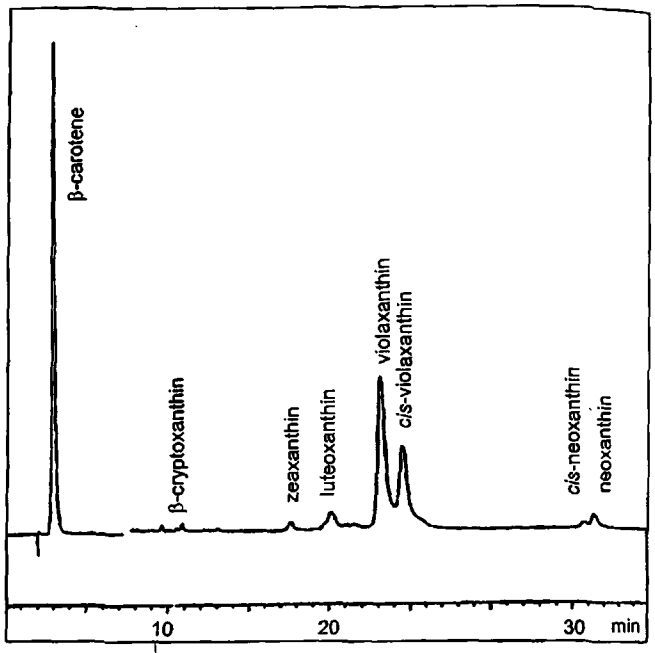
The choice of the most suitable chromatographic method depends on:

- complexity of the carotenoid composition of the sample;
- amount of sample, i.e. CC or large number of TLC plates for large amounts and TLC or HPLC for samples containing < 1 mg of total carotenoid;
- objective of analysis, e.g. for quantitative analysis CC or HPLC, since it is very difficult to totally recover the carotenoids from the TLC plates;
- resolution, purity and speed required;
- equipment available.

Chromatography is essentially a method of separation based on two phases, one stationary and one mobile. If the composition of the mobile phase is not changed during the separation, the term isocratic elution is used. For separation of complex mixtures with wide range of polarities, the composition of the mobile phase can be changed during separation, which is known as gradient elution. There is a large range of stationary phases available, and according to their polarity they can be divided into normal-phase and reversed-phase.

Normal-phase. Silica-gel and aluminum oxide are used to separate the carotenoids according to their polarity, compounds with more polar substituents being more strongly adsorbed. Both are commonly used as stationary phases for separation of carotenoids by CC and/or TLC. The bonded-phase nitrile (or cyano) also separates according to polarity and is employed for separation by HPLC. Figure 2 shows an example of the separation of mango carotenoids on a cyano column with gradient elution. As expected for normal phase, ß-carotene elutes first, followed by mono-, di- and poly-hydroxy carotenoids (12).

FIGURE 2
HPLC separation of carotenoids from mango extract



Conditions: Nitrile Spherisorb column (5µm, 4.6 x 150 mm) and as mobile phase acetone in n-hexane from 0 to 15% in 10 min, to 20% in 20 min, to 30% in 10 min and to 40% in 2 min at 1 ml/ min. Reference: Mercadante et al. (8).

Basic materials such as magnesium oxide (MgO) and calcium carbonate have affinity for conjugated double-bonds, polarity being less important. Thus, a greater number of conjugated double bonds implies stronger retention. The separation of α-carotene and ß-carotene can easily be achieved by CC or TLC using this kind of material (13,14). CC on MgO is also very useful for removing large amounts of colorless impurities from the sample.

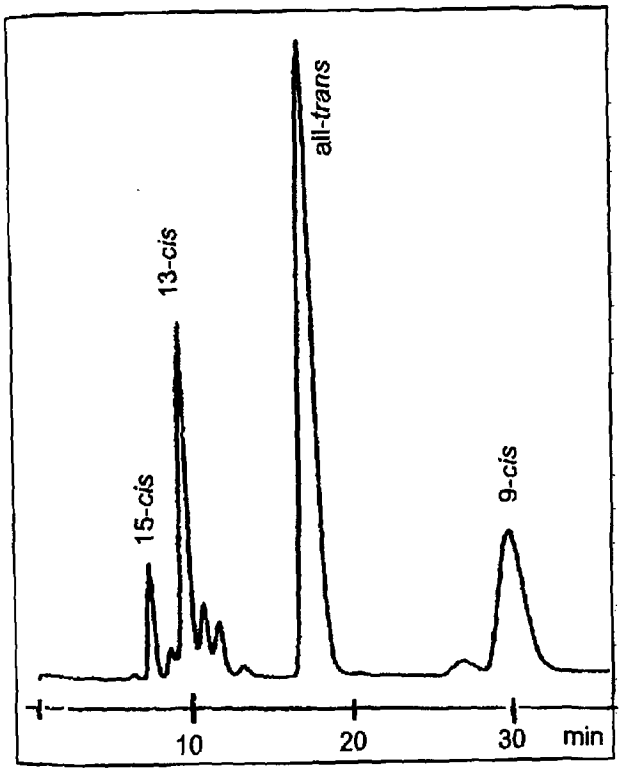
Calcium hydroxide ($Ca(OH)_2$) has been used to separate isomeric forms of ß-carotene (15,16) and ß-cryptoxanthin (16) by CC. Although not commercially available, this stationary phase was also employed in HPLC for separation of isomers of α-carotene, ß-carotene (Figure 3), ζ-carotene and γ-carotene (17).

The isolation of carotenoids from tomato (18-20), mango (12) and passion fruit (21) was successfully carried out using the above stationary phases for the purpose of identification by direct insertion in electron impact-mass spectrometry. Firstly, the saponified extract was separated by CC on alumina, giving three broad fractions (carotenes, mono- and polyhydroxy carotenoids), each of which was separated by TLC on silica, developed with petroleum ether, petroleum ether/diethyl ether (1:1 or 3:2), and diethyl ether according to increasing polarity of the fractions. Each fraction isolated from silica was further

purified by TLC on MgO/kieselguhr with combinations of acetone and petroleum ether as mobile phase.

The appropriate combination of such normal-phase adsorbents can also be employed to obtain standards for HPLC from natural sources.

FIGURE 3
HPLC separation on $Ca(OH)_2$ column of isomers from an isomerized solution of ß-carotene

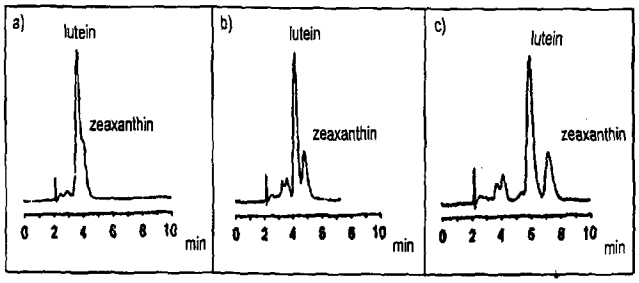


Conditions: $Ca(OH)_2$ (500mesh, 4.6 x 250 mm) column and as mobile phase 2% of *p*-methyl anisole in hexane at 0.7 ml/min. Reference: Schmitz et al. (17).

Reversed-phase. Nowadays, the reversed-phase material is the most popular for separation of carotenoids by HPLC, C_{18} bonded phase being the most employed. Many different C_{18} materials are available from different manufacturers. The difference lies in the:

- degree of carbon loading and end-capping. Silanol groups are expected to influence the retention behavior of polar compounds to a greater extent than nonpolar carotenes. In fact, the separation of α-carotene, ß-carotene and lycopene was little affected by endcapping since the seletivity is unrelated to polarity. On the other hand, the separation of the polar carotenoids lutein and zeaxanthin was influenced by silanol activity and better separation was achieved with the non-endcapped phase (22) (Figure 4).

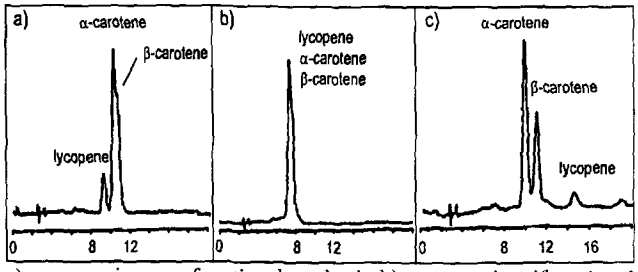
FIGURE 4
Effect of endcapping on polymeric C_{18} phases for separation of lutein and zeaxanthin



a) polymeric endcapped with hexamethyldisilazane, b) polymeric endcapped with trimethylchlorosilane c) polymeric not endcapped. Mobile phase: methanol at 1.5 ml/min. Reference: Sander et al. (22).

- nature of synthesis (monomeric - monofunctional or trifunctional or polymeric). Poorer resolution was observed for α-carotene, ß-carotene and lycopene using the trifunctional monomeric C_{18} phase and nearly baseline separation was achieved with the polymeric one (Figure 5). The polymeric synthesis improves the column seletivity towards form and groups with similar structure (22).

FIGURE 5
HPLC separation on different C_{18} phases of a carotene mixture



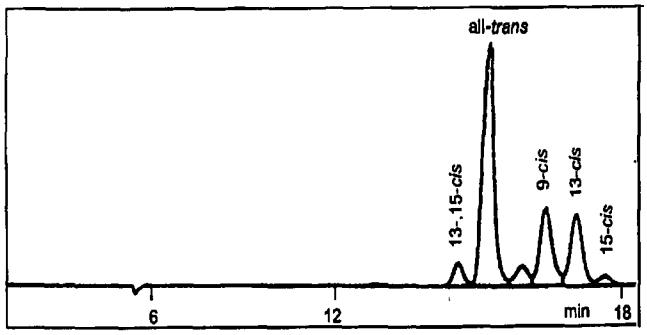
a) monomeric, monofunctional synthesis, b) monomeric, trifunctional synthesis and c) polymeric synthesis. Mobile phase: methanol/ethyl acetate (8:2) at 1.5 ml/min. Reference: Sander et al. (22).

The polymeric C_{18}, with the brand name Vydac, among more than 20 commercial reversed-phase columns, was also the only one that provided the separation of ß-carotene isomers (23). An example of the separation of all-*trans*-, 13,15-di*cis*, 9-*cis*-, 13-*cis* and 15-*cis*- isomers of ß-carotene can be seen in Figure 6. In this case the best separation was achieved at 30°C and resolution was strongly dependent on the column lot (24). The Vydac column has been widely employed for separation of carotenoids in processed foods such as carrot juice (25) and green vegetables (14, 26).

More recently, a C_{30} polymeric reversed-phase column was specially developed for carotenoids (22). This stationary phase shows adequate retention times for polar carotenoids and superior selectivity towards polar and nonpolar carotenoids, specially geometrical isomers. Among reversed-phase

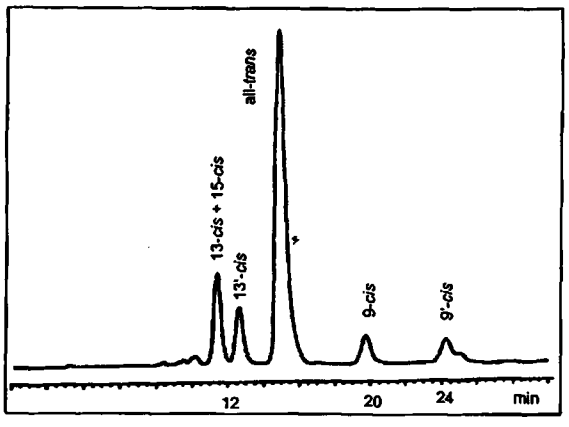
columns, the C_{30} is the only one that was capable of resolving geometrical isomers of asymmetrical carotenoids in which *cis* double bonds are present at the same position but at opposite ends of the molecule (27). This feature is shown in Figure 7, where 13-*cis*, 13'-*cis*, 9-*cis* and 9'-*cis* isomers of lutein were separated (28). This column has also been employed for separation of isomers in isomerized solutions of ß-carotene (28) (Figure 8), in processed vegetables and fruits (29) and of 39 carotenoids from orange juice (30).

FIGURE 6
HPLC separation on Vydac column of isomers from an isomerized solution of ß-carotene



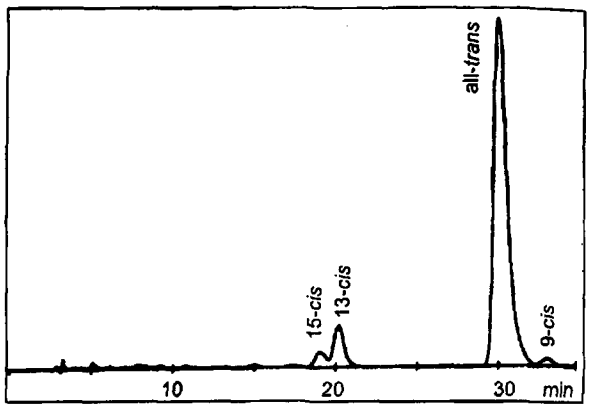
Conditions: C_{18} Vydac 218TP54 (5 µm, 4.6 x 250 mm) column and as mobile phase methanol/tetrahydrofuran (99:1) at 0.6 ml/min. Reference: Schierle et al. (20).

FIGURE 7
HPLC separation on a C_{30} column of isomers from an isomerized solution of lutein



Conditions: C_{30} YMC (3µm, 4.6 x 250 mm) column and as mobile phase methanol/*t*- butyl methyl ether (95:5) at 1 ml/ min. Reference: Brunner (28).

FIGURE 8
HPLC separation on a C_{30} column of isomers from an isomerized solution of ß-carotene



Conditions: C_{30} YMC (3µm, 4.6 x 250 mm) column and as mobile phase methanol/*t*- butyl methyl ether (8:2) at 1 ml/ min. Reference: Brunner (28).

Measurement of NMR spectrum requires very pure compound in higher amount than for UV-visible and mass spectra. For this purpose, the isolation employs CC and/or TLC, and the final purification step is usually carried out by crystallization or semipreparative HPLC. The optimization of HPLC mobile phase is preferably performed in an analytical column with the same characteristics as the semipreparative one. This procedure was used for the isolation and NMR identification of carotenoids from guava (2), annatto (31-33) and saffron (5).

CONCLUSION

Each stationary phase has a different mechanism of separation and therefore the analyst should apply the most suitable phase for a particular separation. A successful carotenoid purification should include chromatographic separation in at least two different kinds of adsorbent. Several examples of carotenoid separation can be found in the literature and it is very useful to look for previous experiences. Different solutions for the same problem may be found in the literature.

In order to enhance reproducibility, authors should provide clear and complete specification of mobile and stationary phases employed for carotenoid separation.

ACKNOWLEDGMENT

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REFERENCES

1. Schiedt K, Liaaen-Jensen S. Isolation and analysis. In: Britton G, Liaaen-Jensen S, Pfander H, editors. Carotenoids vol1A: Isolation and analysis. Basel: Birkhäuser, 1995: 81-108.
2. Mercadante AZ, Steck A, Pfander H. Carotenoids from guava: isolation and structure elucidation. J Agric Food Chem 1999; 47: 145-51.
3. Arima HK, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of commercial Brazilian squashes and pumpkins. J Micronutr Anal 1988; 4: 177-91.
4. Pereira MR, Amaya-Farfan J, Rodriguez-Amaya DB. Avaliação da metodologia analítica para determinação de ß-caroteno em macarrão fortificado. Ciênc Tecnol Aliment 1998; 18: 35-8.
5. Pfister S, Meyer P, Steck A, Pfander H. Isolation and structure elucidation of carotenoid-glycosyl esters in gardenia fruits (*Gardenia jasminoides* Ellis) and saffron (*Crocus sativus* Linne). J Agric Food Chem 1996; 44: 2612-5.
6. Meyer P, Riesen R, Pfander, H. Example 10: carotenoid glycosides and glycosyl esters. In: Britton G, Liaaen-Jensen S, Pfander H, editors. Carotenoids vol1A: Isolation and analysis. Basel: Birkhäuser, 1995: 277-82.
7. Wingerath T, Stahl W, Kirsch D, Kaufmann R, Sies H. Fruit juice carotenol fatty acid esters and carotenoids as identified by matrix-assisted laser desorption ionization (MALDI) mass spectrometry. J Agric Food Chem 1996; 44: 2006-13.
8. Mercadante AZ, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of some native Brazilian green leafy vegetables. Int J Food Sci Technol 1990; 25: 213-9.
9. Mercadante AZ, Rodriguez-Amaya DB. Carotenoid composition of a leafy vegetable in relation to some agricultural variables. J Agric Food Chem 1991; 39: 1094-7.
10. Schiedt K, Bischof S, Glinz E. Metabolism of carotenoids and *in vivo* racemization of (3S,3'S)-astaxanthin in the crustacean *Penaeus*. Meth Enzymol 1993; 214: 148-68.
11. Stewart, I, Wheaton, TA. Conversion of ß-citraurin to reticulaxanthin and ß-apo-8'-carotenal to citranaxanthin during isolation of carotenoids from citrus. Phytochem 1973; 12: 2947-51.
12. Mercadante AZ, Rodriguez-Amaya, DB, Britton, G. HPLC and mass spectrometric analysis of carotenoids from mango. J Agric Food Chem 1997; 45: 120-3.
13. Rodriguez-Amaya DB, Kimura M. Carotenóides e valor de vitamina A em cajá (*Spondias lutea*). Ciênc Tecnol Aliment 1989; 9: 148-62.
14. Godoy HT, Rodriguez-Amaya DB. Occurrence of *cis*-isomers of provitamins A in Brazilian vegetables. J Agric Food Chem 1998; 46: 3081-6.
15. Rodriguez-Amaya DB, Tavares CA. Importance of *cis*-isomer separation in determining provitamin A in tomato and tomato products. Food Chem 1992; 45: 297-302.
16. Godoy HT, Rodriguez-Amaya DB. Occurrence of *cis*-isomers of provitamin A in Brazilian fruits. J Agric Food Chem 1994; 42: 1306-13.
17. Schmitz HH, Emenhiser C, Schwartz SJ. HPLC separation of *cis-trans* carotene isomers using a calcium hydroxide stationary phase. J Agric Food Chem 1995; 43: 1212-8.
18. Britton G, Goodwin TW. The occurrence of phytoene-1,2-oxide and related carotenoids in tomatoes. Phytochem 1969; 8: 2257-8.
19. Britton G, Goodwin TW. Carotene epoxides from the delta tomato mutant. Phytochem 1975; 14: 2530-2.
20. Ben-Aziz A, Britton G, Goodwin TW. Carotene epoxides of *Lycopersicon esculentum*. Phytochem 1973; 12: 2759-64.
21. Mercadante AZ, Britton, G, Rodriguez-Amaya, DB. Carotenoids from yellow passion fruit (*Passiflora edulis*). J Agric Food Chem 1998; 46: 4102-6.
22. Sander LC, Sharpless KE, Craft NE, Wise AS. Development of engineered stationary phases for the separation of carotenoid isomers. Anal Chem 1994; 66: 1667-74.
23. Quackenbush FW. Reverse phase HPLC separation of *cis*- and *trans*-carotenoids and its application to ß-carotenes in food materials. J Liq Chromatogr 1987; 10: 643-53.
24. Schierle J, Härdi W, Faccin N, Bühler I, Schüep, W. Example 8: geometrical isomers of ß,ß-carotene. In: Britton G, Liaaen-Jensen S, Pfander H, editors. Carotenoids vol1A: Isolation and analysis. Basel: Birkhäuser, 1995: 265-272.
25. Chen BH, Peng HY, Chen HE. Changes of carotenoids, color, and vitamin A contents during processing of carrot juice. J Agric Food Chem 1995; 43: 1912-18.
26. Nyambaka H, Ryley J. An isocratic reversed-phase HPLC separation of the stereoisomers of the provitamin A carotenoids (α- and ß-carotene) in dark green vegetables. Food Chem 1996; 55: 63-72.
27. Emenhiser C, Sander LC, Schwartz, SJ. Capability of a polymeric C_{30} stationary phase to resolve *cis-trans* carotenoid isomers in reversed-phase liquid chromatography. J Chromatogr 1995, 707A: 205-16.
28. Brunner MR. Thermische (E/Z)-isomerisierung von carotinoiden. PhD Thesis. University of Berne, 1997: 135p.
29. Lessin WJ, Catigani GL, Schwartz SJ. Quantification of *cis-trans* isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. J Agric Food Chem 1997; 45: 3728-32.
30. Rouseff R, Raley L, Hofsommer HJ. Application of diode array detection with a C-30 reversed phase column for the separation and identification of saponified orange juice carotenoids. J Agric Food Chem 1996; 44: 2176-81.
31. Mercadante AZ, Steck A, Pfander H. Isolation and identification of new apocarotenoids from annatto (*Bixa orellana L.*) seeds. J Agric Food Chem, 1997; 45: 1050-4.
32. Mercadante AZ, Steck A, Pfander H. Isolation and structure elucidation of minor carotenoids from annatto (*Bixa orellana L.*) seeds. Phytochem, 1997; 46: 1379-83.
33. Mercadante AZ, Steck A, Pfander H. Three minor carotenoids from annatto (*Bixa orellana L.*) seeds. Phytochem 1999;52:135-9.

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Sources of errors in the quantitative analysis of food carotenoids by HPLC

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SUMMARY. Several factors render carotenoid determination inherently difficult. Thus, in spite of advances in analytical instrumentation, discrepancies in quantitative results on carotenoids can be encountered in the international literature. A good part of the errors comes from the pre-chromatographic steps such as: sampling scheme that does not yield samples representative of the food lots under investigation; sample preparation which does not maintain representativity and guarantee homogeneity of the analytical sample; incomplete extraction; physical losses of carotenoids during the various steps, especially during partition or washing and by adsorption to glass walls of containers; isomerization and oxidation of carotenoids during analysis. On the other hand, although currently considered the method of choice for carotenoids, high performance liquid chromatography (HPLC) is subject to various sources of errors, such as: incompatibility of the injection solvent and the mobile phase, resulting in distorted or split peaks; erroneous identification; unavailability, impurity and instability of carotenoid standards; quantification of highly overlapping peaks; low recovery from the HPLC column; errors in the preparation of standard solutions and in the calibration procedure; calculation errors. Illustrations of the possible errors in the quantification of carotenoids by HPLC are presented.

Key Words: Carotenoids, quantitative analysis, HPLC, analytical errors.

RESUMEN. Fuentes de errores en el análisis cuantitativo de carotenoides en alimentos por HPLC. Varios factores hacen inherentemente difícil la determinación de carotenoides. A pesar de los avances en la instrumentación analítica, se pueden encontrar en la literatura internacional, discrepancias en los resultados cuantitativos relacionados a los carotenoides. Una gran parte de los errores provienen de las etapas precromatográficas tales como: esquema de muestreo que no produce muestra representativa del lote en estudio; preparación de muestra que no mantenga la representatividad y garantice la homogeneidad de la muestra analítica; extracción incompleta; pérdidas físicas de los carotenoides durante las varias etapas, especialmente durante la partición o lavado y por adsorción en las paredes de vidrios de los recipientes; isomerización y oxidación de los carotenoides durante el análisis. Sin embargo, a pesar de que actualmente el método escogido para análisis de carotenoides es HPLC, éste está sujeto a varias fuentes de errores, tales como: incompatibilidad del solvente de inyección y la fase móvil, resultando en picos distorsionados o divididos; identificación errónea; indisponibilidad, impureza e inestabilidad de los patrones de carotenoides; cuantificación de picos altamente superpuestos; baja recuperación de las columnas de HPLC; errores en la preparación de las soluciones de patrones y en el procedimiento de calibración; errores de cálculo. Se presentan ilustraciones de los posibles errores en la cuantificación de carotenoides por HPLC.

Palabras clave: Carotenoides, análisis cuantitativo, HPLC, errores analíticos.

INTRODUCTION

The day when we can say that most of the data on carotenoid composition of foods are finally reliable is still eluding us. Although there have been tangible strides, and an appreciable part of available analytical information is now reliable, incoherence in published results persists, in spite of the introduction of high performance liquid chromatography (HPLC), currently regarded as the method of choice. A quick look at recent literature illustrates this point, even in terms of only the principal carotenoids. Errors are understandably magnified when minor or trace carotenoids are considered. Tables 1-3 show some recent results on three foodstuffs, obtained in several countries. Lycopene and ß-carotene levels in tomato, from four countries using two analytical techniques, agree well (Table 1), except for the lycopene content of Malaysian tomato. The α-carotene and ß-carotene contents of carrot, obtained in six countries, are more variable, but seems to be mostly a reflection of natural sample variation (Table 2). On the other hand, the carotenoid data for the leafy vegetable *Ipomoea aquatica*, called water spinach by Wills and Rangga (13) of Australia and Hulshof et al. (12) of Indonesia, swamp cabbage by Tee and Lim (4) of Malaysia and water convolvulus by Chen and Chen (11) of Taiwan, are so different that analytical factors must have been involved. Results such as these justify continued strong effort on analytical refinement, so that analytical variability is not mistaken for natural variation of samples.

TABLE 1
Data on principal carotenoids (μg/g) of tomato

Reference, chromatographic technique	Cultivar	ß-Carotene	Lycopene
Hart & Scott (1), UK, HPLC	9 cultivars	4.3-17	12-50
Khachik et al. (2), USA, HPLC	not specified	2.8±0.2	39±1
Tavares & Rodriguez-Amaya (3), Brasil, OCC	Santa Cruz	5.1±1.1	31±20
Tee & Lim (4), Malaysia, HPLC	not specified	3.6	7

HPLC- high performance liquid chromatography; OCC- open column chromatography

TABLE 2
Data on principal carotenoids (μg/g) of carrot

Reference, chromatographic technique	α-Carotene	ß-Carotene
Abdel-Kader (5), Egypt, HPLC	34	63
Chen et al. (6), Taiwan , HPLC	28±3	54±6
Godoy & Rodriguez-Amaya (7), Brasil, OCC	19±1	38±4
Granado et al. (8), Spain, HPLC	29±3	66±0
Hart & Scott (1), UK, HPLC	27 , 36[a]	85 , 108[a]
Heinonen et al. (9), Finland, HPLC	22-49[b]	46-103[b]
Lessin et al. (10), USA, HPLC	39	56
Tee & Lim, Malaysia (4), HPLC	34	68

[a]Two sample lots analyzed in May and September.
[b]19 cultivars.
HPLC- high performance liquid chromatography; OCC- open column chromatography

It is recognized that carotenoid analysis is inherently difficult, the main reasons being: (a) the existence of a large number of naturally occurring carotenoids; (b) the highly variable qualitative and quantitative carotenoid composition of foods; (c) the wide range in concentration of the constituent carotenoids of any given food; and (d) isomerization and degradation of carotenoids during analysis or storage of samples prior to analysis (14-16).

Regardless of the analytical method adopted, a major source of errors is the susceptibility of the highly unsaturated carotenoid molecule to isomerization and oxidation. Thus, special precautions should be taken during analysis, such as: (a) completion of the analysis within the shortest possible time; (b) exclusion of oxygen; (c) protection from light; (d) avoiding high temperature; (e) avoiding contact with acids; (f) use of high purity solvents, free from harmful impurities (e.g. peroxides).

The general procedure in carotenoid analysis consists of: (a) sampling and sample preparation, (b) extraction, (c) partition or transfer to a solvent compatible with the subsequent chromatographic step, (d) saponification and washing, (e) concentration or evaporation of solvent, (f) chromatographic separation, (g) identification and quantification. Evidently, errors can be introduced in each of these steps. Thus, aside from errors arising from the isomerization and oxidation of carotenoids during analysis, other common sources of errors are: (a) analytical samples not representing the food lots under investigation, (b) incomplete extraction, (c) physical losses during the different steps, (d) inefficient chromatographic separation, (e) misidentification, (f) faulty quantification or calculation. Another serious source of error is enzymatic oxidation, which occurs between cutting or disintegration of sample and extraction.

TABLE 3
HPLC data on carotenoids (μg/g) of *Ipomoea aquatica*

Carotenoid	Chen and Chen (11) Taiwan, water convolvulus	Hulshof et al. (12) Indonesia, water spinach	Tee and Lim (4) Malaysia, swamp cabbage	Wills and Rangga (13) Australia, water spinach
ß-Carotene	100±8	27±10	19	4
Cis-ß-carotene	6.8±0.8	4.3±2.2	nd	nd
Lutein	78±7	nd	3.4	6
Violaxanthin	60±5	nd	nd	25
Neoxanthin	50±5	nd	nd	16
Lutein epoxide	29±3	nd	nd	nd
Cis-lutein	11±1	nd	nd	nd
Zeaxanthin	nd	nd	nd	5

HPLC- high performance liquid chromatography; nd- not determined.

Errors in the pre-chromatographic steps

Errors incurred in the steps preceeding chromatography may surpass chromatographic errors and will not be compensated for, no matter how modern and sophisticated the analytical instrumentation may be. In a series of European interlaboratory studies (17), the preliminary conclusion was that preparation of the carotenoid extract for HPLC might account for about 13% of the overall variance of around 23%.

In the interlaboratory studies mentioned above, the same homogenous and stable vegetable mix was analyzed by the different laboratories, thus sampling and sample preparation were not part of the investigation. However, these two initial steps in the analytical process could be major sources of errors.

Several factors markedly influence the carotenoid composition of foods: (a) cultivar or variety; (b) part of plant analyzed; (c) stage of maturity; (d) climate or geographic site of production; (e) harvesting and postharvest handling; and (f) processing and storage. Thus, representative sampling and sample preparation are critical and difficult operations, which, unfortunately, are not well focalized in the carotenoid field. Referring to food analysis in general, Rund (18) eloquently writes, "Are we conscious that the magnitude of sampling errors often exceed three-fold those of the analysis? Why should we be so enamored of new, extremely expensive, and highly sensitive laboratory instrumentation with miraculous detectability characteristics when the gross sample from which the laboratory portion has been extracted was possibly obtained with antiquated equipment and procedure often neither based on scientific fact nor trained personnel?"

Because of the influencing factors cited above, aside from insuring representative sampling and sample preparation, pertinent information must accompany analytical results, such as origin, cultivar, part of plant analyzed, stage of maturity, postharvest handling conditions.

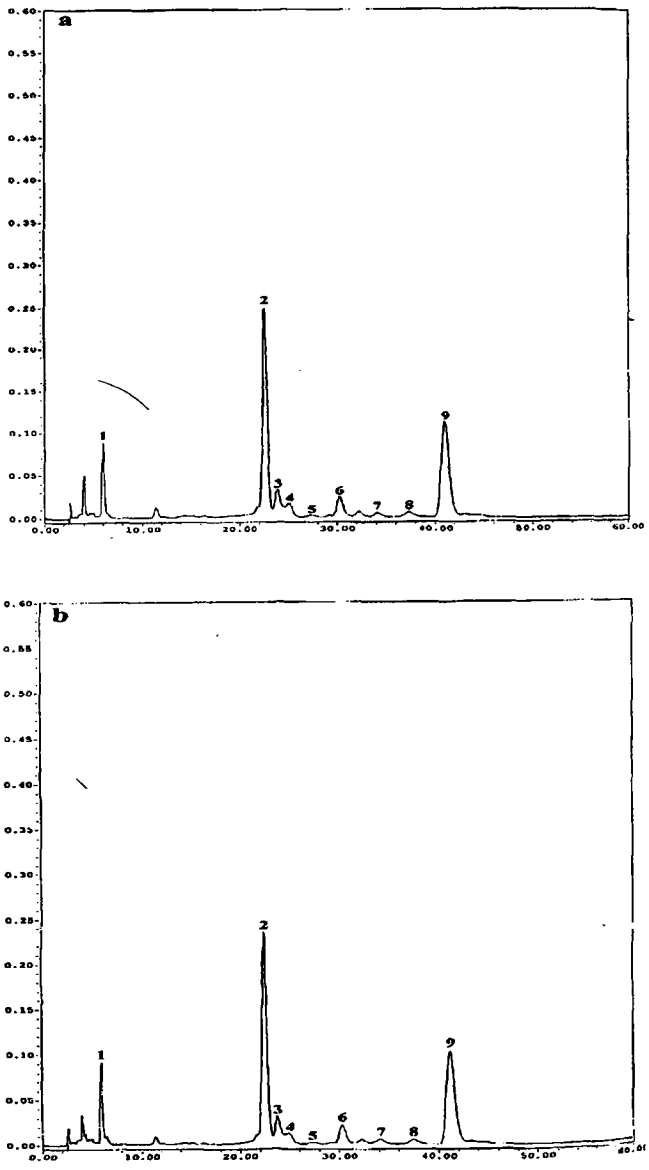
Because of the varying nature of food matrices, including the degree of natural protection conferred on carotenoids, incomplete extraction may be a more common source of error than presently acknowledged. Physical losses, including that resulting from tight adherence of carotenoids in concentrated solutions on the glass walls of containers, are also often overlooked.

The addition of $MgCO_3$ and other neutralizing agent is often done to neutralize the acids liberated from the sample during tissue disintegration to prevent isomerization and degradation. In our laboratory, keeping the time lag between sample maceration and extraction as short as possible, not only prevents enzymatic oxidation, but also makes the addition of $MgCO_3$ unnecessary. No significant difference in the carotenoid concentrations of tomato, an acidic sample, and kale, were observed with or without the addition of $MgCO_3$ (19).

It could be argued that the effect of $MgCO_3$, under the conditions described above, might not be perceptible in terms of the concentration of the constituent carotenoid, but could be seen in terms of isomerization. The chromatogram of the carotenoids of tomato, obtained with or without the use of $MgCO_3$ (Figure 1) are identical and does not support this contention, no *cis*-isomers of ß-carotene being detected in unneutralized tomato with the Vydac column which is capable of separating these geometrical isomers.

FIGURE 1

HPLC chromatograms of tomato extracts obtained with (a) and without (b) the use of $MgCO_3$. Conditions - Column: Spherisorb ODS 5 µm, 2.0x250 mm. Mobile phase: acetonitrile:methanol:ethyl acetate 73:20:7. Flow rate 0.25 mL/min.



Peak identification: 1-lutein, 2-*trans*-lycopene, 3,4-*cis*-lycopene, 5-neurosporene, 6- γ-carotene, 7- *cis*-ζ-carotene, 8-*trans*-ζ-carotene, 9- ß-carotene.

Possible losses during saponification have received more attention. This steps is carried out to remove chlorophylls and unwanted lipids and to hydrolyze carotenol esters, thus simplifying the chromatographic separation, identification and quantification of the carotenoids. However, artefact formation and degradation of carotenoids can occur, the extent of which depends on the carotenoid present and on the saponification conditions (20). The provitamin A carotenoids α-carotene, ß-carotene, γ-carotene and ß-cryptoxanthin can resist saponification (19, 20), but xanthophylls such as lutein, violaxanthin and other dihydroxy and trihydroxy carotenoids can suffer considerable losses (20-22). Thus, saponification should be omitted whenever possible (e. g. analyses of leafy vegetables, tomatoes and carrots) and when indispensable, mild conditions should be used. Saponification of carotenoids dissolved in petroleum ether with an equal volume of 10% KOH overnight at room temperature in the dark, preferably with the addition of BHT and under an atmosphere of N_2, has been generally found to be adequate in our laboratory. Care should also be taken during the subsequent washing as xanthophylls can be easily lost with the water.

Concern about losses of carotenoids has recently led researchers to shorten the time of ambient saponification (1,2, 23). However, complete hydrolysis of carotenoid esters from papaya and *Cucurbita maxima* cultivar Exposição was found to be complete only after overnight saponification (Figures 2 and 3).

Errors in the chromatographic step

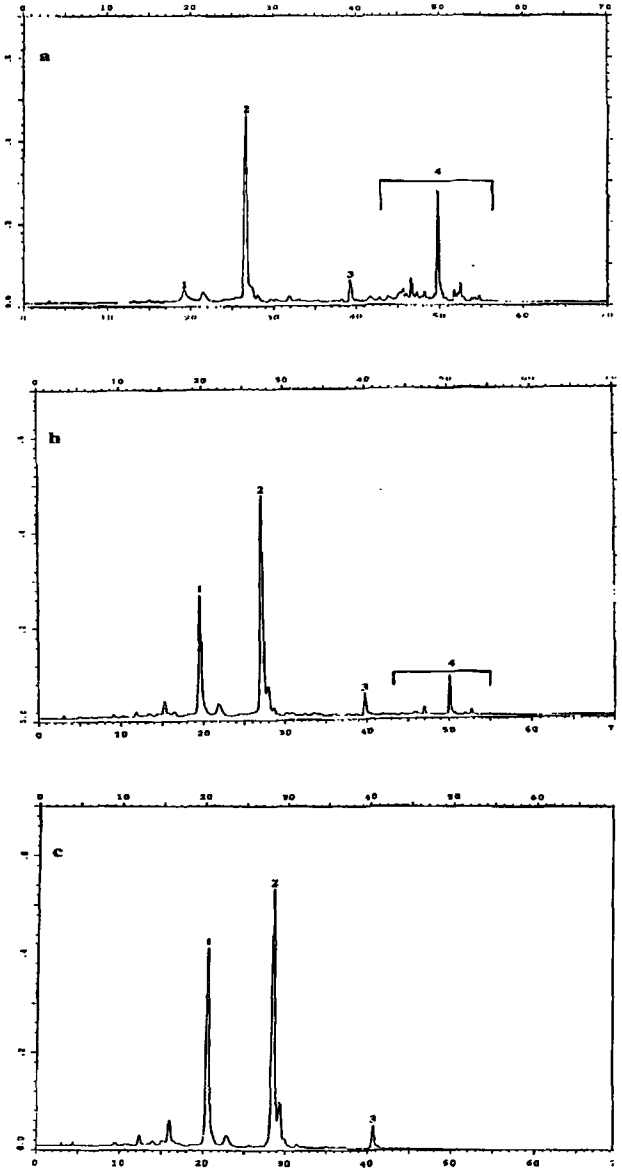
Before carrying out an expensive and complicated analysis, the analyst must clearly define what information is desired. Carotenoid analysis has been carried out at three different levels. For a long time, quantitative analysis of carotenoids involved mainly the determination of the concentrations of only the principal provitamin A carotenoids. With the recognition that vitamin A inactive carotenoids can also be biologically active, determination of major carotenoids, provitamins A or not, have been increasingly carried out. The complete carotenoid composition is the ultimate aim of carotenoid analysis. However, considering that the carotenoid composition of foods typically consist of 1 to 4 principal carotenoids, with a series of carotenoids in minute or trace amounts, it is questionable whether the added information is well worth the greater complexity of the analysis, with greater possibility of errors, higher cost and longer analysis time. In our opinion, the determination of the major carotenoids is adequate for the generation of data for food composition databases.

Although the preferred method for the chromatographic separation of carotenoids, HPLC is subject to several sources of errors: (a) incompatibility of the injection solvent and the mobile phase, (b) erroneous identification, (c) impurity and instability of carotenoid standards, (d) quantification of highly overlapping peaks, (e) low recovery from the HPLC column, (f) errors in the preparation of standard solutions and in the calibration procedure, and (g) erroneous calculation.

The injection solvent must be capable of dissolving all the sample's carotenoids and must also be compatible with the mobile phase. If the injection solvent is much stronger than the mobile phase, the carotenoids can precipitate in the mobile phase, resulting in band broadening and double or tailing peaks, especially when the extract is concentrated (24). On the other hand, a weak injection solvent will not dissolve the carotenoids completely.

FIGURE 2

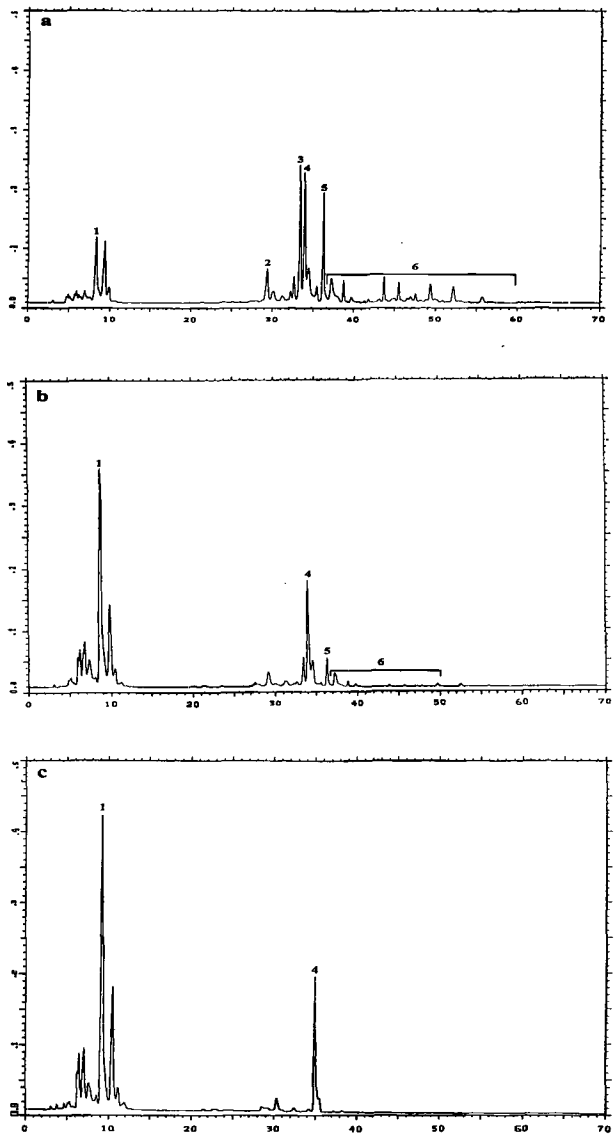
HPLC chromatograms of papaya extracts, unsaponified (a), saponified for 4 hours (b) and saponified overnight (c). Conditions - Column: Novapak 4 μm, 3.9x300 mm. Mobile phase: acetonitrile:methanol:dichoromethane, linear gradient of 80:20:0 to 65:20:15 in 30 min and to 40:20:40 in 20 min. Flow rate: 0.70 mL/min.



Peak identification: 1- ß-cryptoxanthin, 2- lycopene, 3- ß-carotene, 4- esters

FIGURE 3

HPLC chromatograms of *Cucurbita maxima* cultivar Exposição extracts, unsaponified (a), saponified for 4 hours (b) and saponified overnight (c). Conditions - Column: Novapak 4 μm, 3.9x300 mm. Mobile phase: acetonitrile: methanol: dichoromethane, linear gradient of 80:20:0 to 65:20:15 in 20 min and to 40:20:40 in 20 min. Flow rate: 0.70 mL/min.



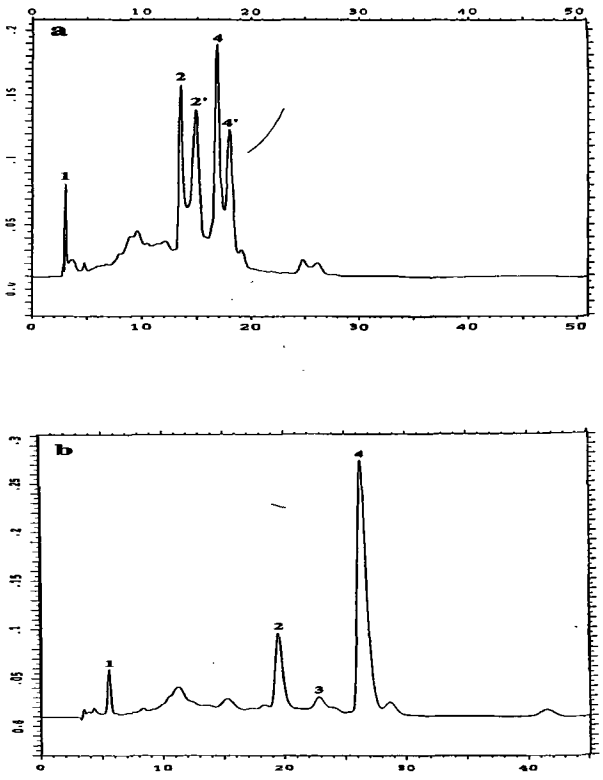
Peak identification: 1- lutein, 4- ß-carotene, 2,3,5 and 6- esters.

Khachik et al. (25) reported peak splitting when carotenoids were injected in dichloromethane, chloroform, tetrahydrofuran, benzene or toluene with a monomeric C_{18} column and a mobile phase consisting of a mixture of acetonitrile, methanol, dicloromethane and hexane. No such splitting occurred when the injection solvent was acetone, acetonitrile, methanol or hexane. On the other hand, Zapata and Garrido (26) observed distorted peaks, especially with the first peaks, when carotenoids were injected in 90% acetone with a gradient of 100% methanol to methanol-acetone (8:2) as mobile phase. No peak distortion was observed when the same extract was injected in 95% methanol or 69% acetone.

As with Khachik et al.(25) and Lietz and Henry (27), in our laboratory, acetone has been found to be a suitable injection solvent. With a Vydac C_{18} polymeric column and a mobile phase of methanol-tetrahydrofuran (95:5), peak splitting occurred when tomato extract was injected in hexane (Figure 4a). However, well defined peaks were obtained when the extract was injected in acetone (Figure 4b). Since occurrence of peak distortion and splitting depends on the chromatographic system used, and results of different laboratories diverge somewhat, the analyst should test his own system.

FIGURE 4

HPLC chromatograms of tomato extracts injected in hexane (a) and in acetone (b). Conditions - Column: Vydac 218 TP54, 5 μm, 4.6x250 mm. Mobile phase: methanol:tetrahydrofuran 95:5. Flow rate: 0.80 mL/min.



Peak identification: 1- lutein, 2,2'- ß-carotene, 3- γ-carotene, 4,4'- lycopene.

According to Craft (24), stronger, miscible solvents can be used as injection solvent if the volume is small (≤ 10 μL) and

the concentrations of the carotenoids are not greatly in excess of their solubility in the mobile phase. In fact, Khachik et al. (25) observed that HPLC peak distortion of carotenoids that occurred with injection solvents such as methylene chloride, choloroform, THF, benzene and toluene, could be eliminated if the injection volume of samples in these solvents were reduced to 5-10 µL. Hexane, which resulted in peak splitting of ß-carotene at higher injection volumes, did not do so at an injection volume of 20 µL. In our system, however, peak splitting was seen with hexane even with an injection volume of 10 µL (Figure 4 a).

Porsch (28) observed that anomalous peaks may be formed, even when sample solubility in the mobile phase is sufficient, if the injection solvent and the mobile phase differ substantially in viscosity and/or the injection solvent strength is considerably higher. He suggested that the viscosity ratio should be kept fairly below two and too high elution power of the injection solvent should be decreased by mixing with the mobile phase prior to injection.

After the introduction of HPLC in the carotenoid field, reversed-phase HPLC C_{18} column immediately became the preferred mode. Among the reasons for such popularity is the weak hydrophobic interaction between the carotenoids and the stationary phase, expected to be less destructive than polar forces in normal-phase chromatography. It was later shown, however, that low recovery of carotenoids from the reversed-phase HPLC column can occur.

Epler et al. (29) investigated the effects of mobile phase, type of stationary phase and the column frit material on recovery of seven carotenoids from sixty commercially available and five experimental HPLC columns. All except five columns were C_{18}. On the average, monomeric C_{18} columns yielded higher recoveries than polymeric C_{18} columns, but were unable to resolve lutein and zeaxanthin. On almost all columns tested, using methanol or methanol-based solvents provided higher recoveries of carotenoids than acetonitrile or acetonitrile-based solvent (Table 4). Recovery with acetonitrile-based solvents was improved with the addition of ammonium acetate and triethylamine, an observation later confirmed by Hart and Scott (1).

TABLE 4
Average recovery of carotenoids with different mobile phases

Mobile phase	Number of columns tested	Recovery ± SD (%)[a]
100% methanol	29	84±8
Methanol-tetrahydrofuran	35	86±11
Methanol-ethyl acetate	35	82±12
100% acetonitrile	21	56±19
Acetonitrile-tetrahydrofuran	43	68±17
Acetonitrile-ethyl acetate	43	47±17

[a]Mean and standard deviation
Reference:Epler et al.(29)

Recovery was also found by Epler et al. (29) to be dependent on the carotenoid structure. Losses of zeaxanthin and ß-carotene, both having two ß-rings, were greater than those of lutein and α-carotene, both containing one ß- and one ε-ring. Within the group of ß,ß-carotenoids, recovery increased as polarity decreased. Recovery increased in the following order: zeaxanthin (dihydroxy) < ß-cryptoxanthin (monohydroxy) < echinenone (monoketo) < ß-carotene. For the two ß,ε-carotenoid, the recovery of lutein (dihydroxy) was less than that of α-carotene. Hart and Scott (1) also found differences in the recovery of individual carotenoids, suggesting that on-column losses varied with different carotenoids.

Although recoveries were slightly lower for stainless steel frits, Epler et al. (29) observed no significant difference in recovery in using stainless steel, titanium or "biocompatible" (hastealloy) frits. Degradation of carotenoids provoked by the metal surface of stainless steel frits of the guard and analytical column was, however, reported by several authors in recent years (23,30,31). Thus, the use of the "biocompatible" hastealloy frits was advocated. But even with this frit, Konings and Roomans (23) observed considerable loss (approximately 40%) of lycopene, leading them to suggest that a PAT (peek alloyed with teflon) frit be used.

The accuracy of HPLC quantification of carotenoids obviously depends on how well the chromatogram peak areas are measured. Especially in earlier HPLC studies, data on food carotenoids have been obtained by quantifying highly overlapping peaks. Although working with non-carotenoid compounds (naphthalene and anthracene), Meyer (32) gave an idea of the magnitude of the error derived from integration of incompletely resolved chromatographic peaks. Errors increased with increasing size ratio of the fused peaks, increasing tailing and decreasing resolution. Within the range of parameters investigated (size ratio up to 10:1, tailing to 2.0, resolution down to 0.75), the relative error can reach a 40% deviation in peak area.

Highly efficient columns are now available, which with judicious choice of mobile phase, can provide good resolution of even complex mixtures, such as carotenoid extracts from foods.

Errors in the identification step

The chromatographic behavior and the UV-visible absorption spectrum are the first tools used to identify carotenoids. The retention time reflects the polarity; and the wavelengths of maximum absorption and the fine structure (shape) of the spectrum reflect the chromophore. However, the use of these two parameters as sole criteria for identification, although a common practice, may not be conclusive and may lead to erroneous identification. Retention times are difficult to reproduce, and even when authentic carotenoids are available for co-chromatography, identification will still be inconclusive since different carotenoids may have the same retention time. Likewise, different carotenoids may have the same

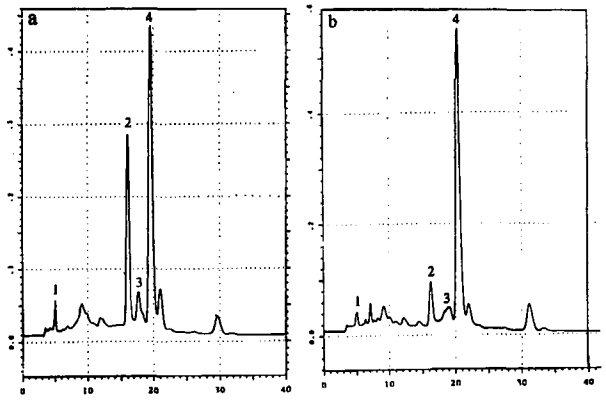
chromophore and thus present the same spectrum. Some examples of misidentifications are given below.

α-Cryptoxanthin and zeinoxanthin both monohydroxy derivatives of α-carotene, differ only in the position of the hydroxy group, thereby presenting identical spectrum and very similar chromatographic behavior. They can be differentiated by simple methylation with acidified methanol, α-cryptoxanthin responding positively because of the allylic position of the hydroxy substituent. Seemingly, these two carotenoids are often confused with each other and even with ß-cryptoxanthin.

With the photodiode array detector, testing the peak purity is easier, avoiding the identification of a peak of a mixture of carotenoids as that of a sole carotenoid. A quick look at the chromatograms in Figure 5 may give the idea that peak 3 in both the fresh tomato and the tomato paste is γ-carotene. The spectra taken at the ascending and descending slopes and at the maximun show that while peak 3 of the fresh tomato was pure γ-carotene, this peak in the tomato paste was a mixture (Figure 6).

FIGURE 5

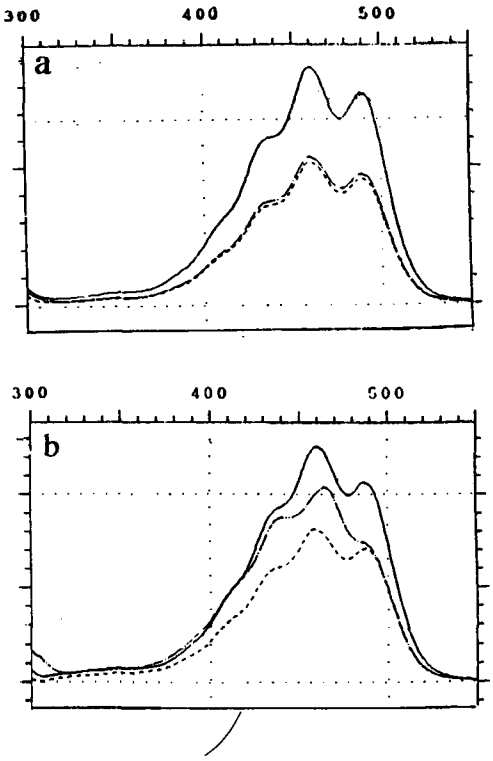
HPLC chromatograms of raw tomato (a) and tomato paste (b). Conditions - Column: Vydac 218 TP54, 5 µm, 4.6x250 mm. Mobile phase: methanol:tetrahydrofuran 95:5. Flow rate: 0.80 mL/min.



Peak identification: 1- lutein, 2- ß-carotene, 3- γ-carotene in raw tomato and mixture in tomato paste, 4- lycopene.

FIGURE 6

Absorption spectra corresponding to peak 3 of Figure 5 obtained with the photodiode array detector of raw tomato (a) and tomato paste (b) at maximum (————), upslope (---------) and downslope (-.-.-.-.).



Unlike fruits and roots, leaves have been known to contain the same principal carotenoids: lutein, ß-carotene, violaxanthin and neoxanthin. Siefermann-Harms et al. (23) showed that lettuce also contains lactucaxanthin. Usually overlooked, lactucaxanthin appears to be present in similar or greater amounts than neoxanthin as shown in Figure 7.

In cases where the judicious and combined use of chromatographic data, co-chromatography with authentic samples, UV-visible absorption spectra and chemical reactions do not yield conclusive identifications, mass spectrometry and nuclear magnetic resonance spectroscopy, two techniques required in structure elucidation, will have to be used.

Errors in the quantification step

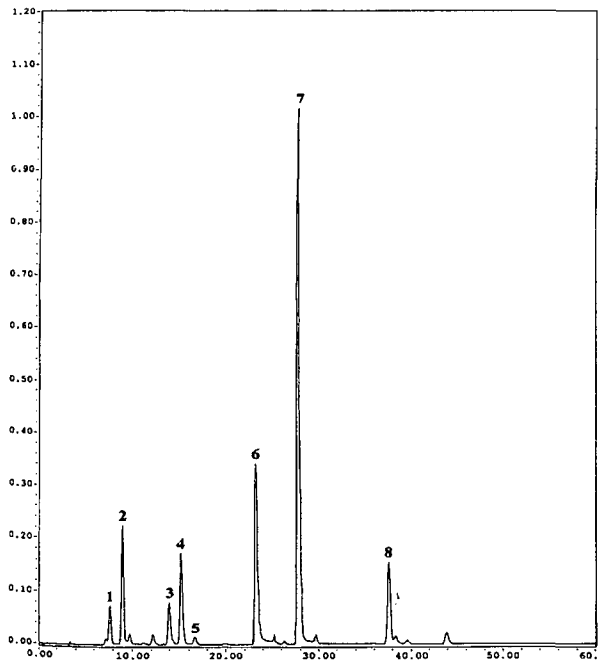
In HPLC, concentrations of the analytes are determined by comparison with standard solutions of known concentrations. Thus, any error in the preparation and quantification of the standard solutions themselves will be directly reflected in the quantitative data obtained.

Quantification of carotenoids is made difficult by the widely varying purity of commercial standards (34, 35), very limited number of carotenoid standards available commercially

and instability of carotenoids. The purity of carotenoid standards should always be verified and impure standards repurified. Instead of repurifying, Hart and Scott (1) assessed the "purity" of the carotenoid by HPLC, and expressed it as the peak area of the carotenoid as a percentage of the total area of the chromatogram. The concentration of the carotenoid standard calculated from the absorbance reading was corrected accordingly. Carotenoids not available commercially, can be isolated from natural sources, but this is an operation that requires skill, experience and care. Although several authors claim stability of carotenoid stock solutions at -18°C under N_2 for an extended period, it is our experience as well as of others (36) that carotenoid standard solutions can only be used over a very short period.

FIGURE 7

HPLC chromatogram of lettuce. Conditions - Column: Spherisorb ODS 3 µm, 4.6x150 mm. Mobile phase: acetonitrile:methanol:ethyl acetate, convex gradient of 95:5 to 60:20:20 in 20 min. Flow rate: 0.50 mL/min



Peak identification: 1- neoxanthin, 2- violaxanthin, 3- lactucaxanthin, 4- lutein, 5- zeaxanthin, 6 and 7- chlorophylls, 8- ß-carotene.

The standard curve should be linear, pass through or very near the origin and must bracket the concentrations of the food samples. To fulfill the third requirement, the analyst will have to work on vastly different ranges since the carotenoid concentrations of a given food vary over a very wide range.

Khachick et al. (37) cited the following parameters to evaluate the validity of the standards and the instrumentation: (a) the correlation coefficient should be greater than 0.9, (b) the intercept should be very close to zero, (c) the relative standard deviation of the regression should be less than 5%. If any of these parameters is out of range, the standard as well as the HPLC instrumentation should be carefully checked and the standard curve rerun. Mantoura et al. (36) recommended a coefficient of correlation greater than 0.95.

Finally, some calculation errors must be involved since, ocasionally, for a certain foodstuff, a laboratory would come up with a value about 10 times those reported by the other laboratories.

In order to limit analytical variability, in the European interlaboratory studies (17), the following measures were taken by the participating laboratories: (a) the spectrometers were calibrated; (b) the same absorption coefficients and absorption maxima were used; (c) a sample extract was circulated for analysis, using circulated and in-house standards, to verify differences in standards; (d) a common data handling approach was used , including the use of peak area instead of peak height.

In closing, it can be said that HPLC is truly a potentially powerful technique. However, it is very easy to make mistakes with this technique and because the results are precise, lack of accuracy easily passes unnoticed. The analyst should guard against undue confidence that modern instrumentation can inadvertently give.

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REFERENCES

1. Hart DJ, Scott KJ. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. Food Chem 1995; 54: 101-11.
2. Khachik F, Goli MB, Beecher GR, Holden J, Lusby WR, Tenorio MD, Barrera MR. Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. J Agric Food Chem 1992; 40: 390-8.
3. Tavares CA, Rodriguez-Amaya DB. Carotenoid composition of Brazilian tomatoes and tomato products. Lebensm Wiss Technol 1994; 27: 219-24.
4. Tee ES, Lim CL. Carotenoid composition and content of Malaysian vegetables and fruits by the AOAC and HPLC methods. Food Chem 1991; 41: 309-39.
5. Abdel-Kader Z.M. Determination of carotenoids in foods by high-performance liquid chromatography. Nahrung 1991; 35: 689-93.
6. Chen BH, Chuang JR, Lin CP, Chiu CP. Quantification of provitamin A compounds in Chinese vegetables by high-performance liquid chromatography. J Food Prot 1993; 556: 51-4.

7. Godoy HT, Rodriguez-Amaya DB. Occurrence of *cis*-isomers of provitamins A in Brazilian vegetables. J Agric Food Chem 1998; 46: 3081-6.
8. Granado F, Olmedilla B, Blanco I, Rojas-Hidalgo E. Carotenoid composition in raw and cooked Spanish vegetables. J Agric Food Chem 1992; 40: 2135-40.
9. Heinonen MI. Carotenoid and provitamin A activity of carrot (*Daucus carota L.*) cultivars. J Agric Food Chem 1990; 38: 609-12.
10. Lessin WJ, Catigani GL, Schwartz SJ. Quantification of *cis-trans* isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. J Agric Food Chem 1997; 45: 3728-32.
11. Chen BH, Chen YY. Determination of carotenoids and chlorophylls in water convolvulus (*Ipomoea aquatica*) by liquid chromatography. Food Chem 1992; 45: 129-34.
12. Hulshof PJM, Xu C, Van de Bovenkamp P, Muhilal, West CE. Application of a validated method for the determination of provitamin A carotenoids in Indonesian foods of different maturity and origin. J Agric Food Chem 1997; 45: 1174-9.
13. Wills RBH, Ranga A. Determination of carotenoids in Chinese vegetables. Food Chem 1996; 56: 551-5.
14. Rodriguez-Amaya DB. Critical review of provitamin A determination in plant foods. J Micronutr Anal 1989; 51: 191-225.
15. Rodriguez-Amaya DB. Provitamin A determination - problem and possible solutions. Food Nutr Bull 1990; 5: 246-50.
16. Rodriguez-Amaya DB, Amaya-Farfan J. Estado actual de los metodos analiticos para determinar provitamina A. Arch Latinoamer Nutr 1992; 42: 180-91.
17. Scott KJ, Finglas PM, Seale R, Hart DJ, Froidmont-Görtz. Interlaboratory studies of HPLC procedures for the analysis of carotenoids in foods. Food Chem 1996; 57: 85-90.
18. Rund RC. The hidden factor. The Referee - Association of Official Analytical Chemists 1991; 15(1):11.
19. Rodriguez-Amaya DB, Kimura M, Godoy HT, Arima HK. Assessment of provitamin A determination by open column chromatography/visible absorption spectrophotometry. J Chromatogr Sci 1988; 26: 624-9.
20. Kimura M, Rodriguez-Amaya DB, Godoy HT. Assessment of the saponifiction step in the quantitative determination of carotenoids and provitamin A. Food Chem 1990;35:187-95.
21. Khachik F, Beecher GR, Whitaker NF. Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. Food Chem 1986; 56: 599-603.
22. Riso P, Porrini M. Determination of carotenoids in vegetable foods and plasma. Internat J Vit Nutr Res 1997; 67: 47-54.
23. Konnings EJM, Roomans. Evaluation and validation of an LC method for the analysis of carotenoids in vegetables and fruit. Food Chem 1997; 59: 599-603.
24. Craft NE. Carotenoid reversed-phase high performance liquid chromatography methods: Reference compedium. Meth Enzymol 1992; 213: 185-205.
25. Khachik F, Beecher GR, Vanderslice JT, Furrow G. Liquid chromatographic artifacts and peak distortion: sample-solvent interactions in the separation of carotenoids. Anal Chem 1988; 60: 807-11.
26. Zapata M, Garrido JL. Influence of injection conditions in reversed-phase high performance chromatography of chrolophylls and carotenoids. Chromatogr 1991; 31: 589-94.
27. Lietz G, Henry CJK. A modified method to minimise losses of carotenoids and tocopherols during HPLC analysis of red palm oil. Food Chem 1997; 60: 109-17.
28. Porsch B. Experimental consequences of differences between composition of the sample solvent and of the mobile phase in HPLC. J Liq Chromatogr 1993; 16: 3409-21.
29. Epler KS, Sander LC, Ziegler RG, Wise AS, Craft NE. Evaluation of reversed-phase liquid chromatographic columns for recovery and selectivity of selected carotenoids. J Chromatogr 1992; 595: 89-101.
30. Craft NE, Wise AS, Soares Jr. JH. Optimization of an isocratic high-performance liquid chromatographic separation of carotenoids. J Chromatogr 1992; 589: 171-6.
31. Scott KJ. Observations on some of the problems associated with the analysis of carotenoids in foods by HPLC. Food Chem 1992; 45: 357-64.
32. Meyer VR. Errors in the area determination of incompletely resolved chromatographic peaks. J Chromatogr Sci 1995; 33: 26-33.
33. Siefermann-Harms D, Hertzberg S, Borch G, Liaeen-Jensen S. Lactucaxanthin, an ε,ε-carotene-3, 3'-diol from *Lactuca sativa*. Phytochem 1981; 20: 85-8.
34. Quackenbush FW, Smallidge RL. Nonaqueous reverse phase liquid chromatography system for separation and quantitation of provitamins A. J Assoc Anal Chem 1986; 69: 767-72.
35. Craft NE, Sander LC, Pierson HF. Separation and relative distribution of all-*trans*-ß-carotene and its *cis* isomers in ß-carotene prepartions. J Micronutr Anal 1990; 8: 209-21.
36. Mantoura RFC, Repeta DJ. Calibration methods for HPLC. In: Jeffrey SW, Mantoura RFC, Wright SW, editors. Phytoplankton pigments in oceanography: guidelines to modern methods. Paris: UNESCO Publishing 1997: 407-28.
37. KhachikF, Beecher GR, Goli MB, Lusby WR. Separation and quantitation of carotenoids in foods. Meth Enzymol 1992; 213: 347-59.

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Regulamentação de uso de corantes naturais

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RESUMO. Vários corantes naturais extraídos de vegetais, insetos e algas são hoje usados em alimentos, embora muitos deles não tenham sido avaliados quanto a sua segurança de uso. Na avaliação toxicológica de aditivos alimentares o conceito de Ingestão Diária Aceitável (IDA) tem sido empregado como indicação de sua segurança de uso, possibilitando que órgãos de regulamentação tomem as medidas legislativas adequadas para seu controle. Neste trabalho serão discutidos os princípios recomendados pelo Comitê Conjunto FAO/OMS de Peritos em Aditivos Alimentares e Contaminantes (JECFA) para a avaliação da segurança dos aditivos alimentares, com ênfase nas orientações estabelecidas para a avaliação de corantes naturais. Dados recentes sobre a ingestão potencial de urucum no Brasil e aspectos atuais da regulamentação de uso de corantes naturais no âmbito do Mercosul serão também abordados.
Palavras-chave: Corantes naturais, aditivos alimentares, regulamentação de uso.

SUMMARY. Regulatory aspects of natural food colours. A number of preparations of natural colours from vegetable, insect and algae sources are presently used in various foods, although many of them have not been evaluated in relation to their safety of use. In evaluating the toxicity of food additives the concept of the Acceptable Daily Intake (ADI) has been used to provide an indication of safety for use and to enable regulatory authorities to take adequate legislative measures for their control. This paper will focus on the principles for the safety assessement of food additives, with emphasis on the guidelines that have been established by the Joint Expert Committee on Food Additives and Contaminants (JECFA) for evaluating natural food colours. Recent data on the potential intake of annato extracts in Brazil and current aspects of regulation of food colours at the level of MERCOSUR will also be presented.
Key words: Natural colours, food additives, regulatory aspects.

INTRODUÇÃO

No Brasil, assim como em outros países, os corantes são utilizados amplamente pela indústria de alimentos, sendo seu emprego limitado por legislações específicas, apoiadas em critérios restritos que levam em consideração recomendações e sugestões emitidas em nível mundial por Comitês de especialistas. Organizações internacionais avaliam regularmente os aditivos alimentares e estabelecem especificações e limites para o seu uso, possibilitando que agências governamentais responsáveis por seu controle regulamentem o emprego de aditivos pela indústria de alimentos.

A Comissão do Codex Alimentarius, um órgão subsidiário da FAO (Organização para Alimentação e Agricultura) e da OMS (Organização Mundial da Saúde) é um fórum para a elaboração de padrões para alimentos envolvidos no comércio internacional e para a orientação de países que desejam criar suas próprias leis e regulamentação para alimentos (1). O Codex trata especificamente da questão do uso seguro de aditivos alimentares através do Comitê do Codex para Aditivos Alimentares e Contaminantes, CCFAC, um de seus vários Comitês subsidiários. Ao estabelecer ou endossar níveis máximos permitidos de aditivos em alimentos, o CCFAC leva em consideração, além de dados sobre necessidade tecnológica e justificativas para níveis de uso propostos, as avaliações toxicológicas e recomendações do Comitê Conjunto FAO/OMS de Peritos em Aditivos Alimentares e Contaminantes (JECFA) (1). O JECFA conduz a avaliação toxicológica dos aditivos alimentares, recomendando, quando possível, valores de ingestão diária aceitável (IDA), fornecendo assim uma indicação de sua segurança para uso em alimentos (2). Na prática, os valores de IDA são usados por agências nacionais e internacionais para estabelecer quantidades aceitáveis de aditivos alimentares a serem utilizados em diferentes alimentos, de forma a que seu consumo não exceda as IDAs recomendadas.

No âmbito da Organização Mundial do Comércio (OMC), os acordos sobre aplicação de Medidas Sanitárias e Fitossanitárias - SPS/OMC - e de Barreiras Técnicas ao Comércio -TBT/OMC- especificam que em temas relacionados à Segurança e Qualidade de Alimentos devem ser seguidas as Normas, Orientações e Recomendações da Comissão do Codex Alimentarius (3). Desta forma, as decisões e recomendações do Codex tem servido de base para estabelecimento de legislações nacionais e de blocos econômicos, entre eles o MERCOSUL

Regulamentação de uso de corantes em alimentos

Independente de sua classe funcional, a aprovação do emprego de aditivos em alimentos deve atender a critérios

específicos estabelecidos por organismos de regulamentação. O uso do aditivo deve ser justificado tecnologicamente e sua segurança comprovada, não apresentando riscos para a saúde do consumidor nos níveis propostos de uso.

No caso específico de corantes, existem várias justificativas para a necessidade de sua adição em alimentos. Alguns alimentos perdem sua cor durante o processamento e/ou estocagem e a adição de cor compensa esta perda. Existe também uma preferência do consumidor ou tradições culturais por alimentos com uma cor característica. Além disto, os corantes são adicionados a alimentos para melhorar sua aparência e aumentar sua aceitabilidade, dando uma cor distinta a alguns alimentos processados incolores ou proporcionando uniformidade na cor do alimento.

Quanto à segurança de uso, são exigidos dados de avaliação toxicológica e informações sobre a ingestão provável do corante decorrente dos usos propostos.

Avaliação toxicológica

A avaliação toxicológica de um aditivo visa determinar o seu potencial tóxico e a dose que o evidencia. O processo envolve basicamente dois estágios principais: a investigação toxicológica e a avaliação toxicológica propriamente dita, com a interpretação dos dados obtidos e sua extrapolação para o homem

O JECFA, órgão científico consultor da FAO e da OMS, conduz a avaliação toxicológica dos aditivos alimentares, fornecendo uma indicação de sua segurança para uso em alimentos (2). Este Comitê, adotado como organismo internacional de referência no âmbito do Mercosul (4), têm estabelecido, com base em dados científicos adequados, ingestões diárias aceitáveis para os aditivos avaliados. Os membros do JECFA são cientistas independentes, com representatividade geográfica, selecionados pela competência e experiência. Os membros do JECFA convidados pela Organização Mundial da Saúde são responsáveis pelo estabelecimento de princípios para avaliação e ensaios toxicológicos, pela revisão de dados toxicológicos e pelo estabelecimento da Ingestão Diária Aceitável (IDA) para aditivos e contaminantes. Os membros do JECFA convidados pela FAO são, por sua vez, responsáveis, entre outros, pelo estabelecimento de especificações para identidade e pureza de aditivos alimentares e pelo estabelecimento de limites máximos de resíduos (LMR) para drogas veterinárias.

Para alguns grupos de aditivos, entre eles os corantes naturais, foram estabelecidas pelo JECFA orientações para sua avaliação toxicológica (2), devendo o corante ser enquadrado dentro de três grupos principais:

- corante não modificado quimicamente isolado de um alimento conhecido, e usado no alimento do qual foi extraído em níveis normalmente encontrados naquele alimento. Este corante poderá ser aceito da mesma forma que o próprio alimento, sem exigência de dados toxicológicos.
- corante não modificado quimicamente, isolado de um alimento conhecido, e usado em níveis acima dos normalmente encontrados naquele alimento, ou usado em outros alimentos além daquele do qual foi extraído. Poderão ser exigidos dados toxicológicos normalmente requeridos para avaliar a toxicidade de corantes sintéticos.
- corante isolado de um alimento conhecido e modificado quimicamente durante sua produção, ou corante natural isolado de uma fonte que não é alimento. Este produto requer dados toxicológicos normalmente exigidos para corantes sintéticos.

O JECFA reconhece que corantes naturais podem ser reproduzidos por síntese química, porém considera que corantes idênticos aos naturais produzidos por síntese química podem conter impurezas, devendo ser avaliados da mesma forma que corantes sintíticos, cuja avaliação toxicológica exige os seguintes dados mínimos (2):

- estudos metabólicos em várias espécies, incluindo de preferência o Homem. Devem ser incluídos estudos de absorção, distribuição, biotransformação e eliminação.
- estudos a curto prazo em mamífero não roedor.
- estudos de reprodução e teratogênese em várias gerações.
- estudos de toxicidade a longo prazo / carcinogenicidade em duas espécies

Em várias oportunidades, o JECFA tem manifestado dificuldades na avaliação toxicológica de corantes naturais, devido principalmente à escassez de informação sobre seu metabolismo e toxicidade, e ausência de especificação adequada.

Corantes naturais normalmente contêm mais do que um e, em geral, vários componentes coloridos e outros mais. Sua composição varia conforme a fonte e o método de preparo, e mesmo a composição de componentes da mesma espécie da mesma planta pode variar devido a condições climáticas, idade da planta, tempo da colheita, etc. (5). Muitos corantes naturais são mistura de substâncias quimicamente relacionadas, o que dificulta ainda mais a interpretação dos dados toxicológicos. Desta forma, especificações adequadas quanto ao material de origem, método de preparação e composição química do extrato colorido são informações imprescindíveis para a avaliação toxicológica de corantes naturais.

Cálculo da ingestão diária aceitável

A interpretação dos dados de ensaios toxicológicos conduzida pelo JECFA, identifica, quando possível, uma dose experimental na qual não tenham sido observados efeitos adversos da substância avaliada sobre a espécie animal mais sensível (2). Esta dose, conhecida como NOEL (no observed effect level), e expressa em mg/kg peso corpóreo, é utilizada para a extrapolação dos resultados dos estudos com animais experimentais para o homem, através da aplicação de um fator de segurança arbitrário. Este fator procura considerar, entre

outros, diferenças de sensibilidade entre espécies e a heterogenicidade da população humana (6). Um fator de segurança igual a 100 tem sido largamente aceito para aditivos alimentares e é bastante utilizado pelo JECFA.

O valor numérico extrapolado para o homem, denominado de ingestão diária aceitável (IDA), representa a quantidade de um aditivo, expressa em mg/kg de peso corpóreo, que se pode consumir diariamente e por toda a vida, sem risco apreciável à saúde, à luz dos conhecimentos toxicológicos disponíveis na época da avaliação (7,8). O conceito de IDA se baseia na premissa de que todas as substâncias químicas são tóxicas, mas que suas toxicidades variam quanto à natureza do efeito e à quantidade que é necessária para produzir sinais e sintomas tóxicos. Valores de IDA são atribuídos somente àquelas substâncias que apresentam um dossiê toxicológico completo, preparado de acordo com protocolos e exigências pré-estabelecidos.

Em função dos dados toxicológicos disponíveis, diferentes categorias de IDA podem ser atribuídas aos aditivos, a saber (8,9):

- IDA numérica: as investigações são consideradas completas e um valor numérico de IDA é recomendado.
- IDA não especificada: em face às informações toxicológicas disponíveis sobre o aditivo e ao seu emprego de acordo com a boas práticas de fabricação, o estabelecimento de um valor numérico para a IDA é considerado desnecessário.
- IDA temporária: atribuída por um período limitado de tempo, até que se concluam os estudos toxicológicos exigidos. Neste caso, um fator de segurança superior a 100, em geral 200, é aplicado quando se extrapolam para o homem os resultados obtidos com animais de laboratório.
- IDA não alocada: os dados toxicológicos disponíveis não são suficientes para se estabelecer a segurança de uso do aditivo.
- IDA de grupo: recomendada para um grupo de compostos que apresentam os mesmos efeitos tóxicos, evitando assim uma ingestão acumulativa.

Existem ainda situações nas quais o uso do aditivo é considerado aceitável apenas sob determinadas condições de uso.

Na Tabela 1, são apresentados valores de IDA recomendados pelo JECFA para corantes do grupo dos carotenóides (9). Os números em parentêses referem-se ao ano em que foi conduzida a avaliação.

Para fins de comparação, a Tabela 2 apresenta valores de IDA de corantes artificiais (9) aprovados no âmbito do MERCOSUL.

Estimativas de ingestão provável

A Comissão do Codex Alimentarius identificou em sua 22a. Reunião (10) três principais componentes da análise de riscos: avaliação do risco, gerenciamento do risco e comunicação do risco, e incorporou às suas atividades quatro princípios relativos ao papel da avaliação de risco na segurança de alimentos. Entre as etapas envolvidas no processo de avaliação de risco está a avaliação da exposição, definida como a avaliação qualitativa e/ou quantitativa da ingestão provável de agentes biológicos, químicos e físicos através dos alimentos, assim como as exposições que derivam de outras fontes (1).

TABELA 1
Ingestão diária aceitável (IDA) de corantes naturais

Corante	IDA (mg/kg p.c.)
Carotenos naturais (vegetais)	aceitável (1993)*
Urucum (bixina)	0-0,065 (1982)
ß-Caroteno sintético	0-5 (1974)
Cantaxantina	0-0,03 (1995)
Capsantina	não alocada (1989)**
Licopeno	não avaliado

*desde que o nível de uso não exceda o nível normalmente encontrado em vegetais.
**auto limitante como tempero

TABELA 2
Ingestão diária aceitável (IDA) de corantes artificiais

Corante	IDA (mg/kg p.c.)
Amarelo crepúsculo	0-2,5 (1982)
Tartrazina	0-7,5 (1964)
Azul patente V	não alocada (1982)
Azul brilhante	0-12,5 (1969)
Indigotina	0-5 (1974)
Amaranto	0-0,5 (1984)
Eritrosina	0-0,1 (1990)
Ponceau 4R	0-4 (1983)
Vermelho 40	0-7 (1981)
Verde rápido FCF	0-25 (1986)
Azorrubina	0-4 (1983)

Para atender às novas recomendações do Codex, encontra-se em discussão no âmbito do Comitê do Codex para Aditivos Alimentares e Contaminantes (CCFAC) a integração formal da análise de riscos às atividades de desenvolvimento de normas para aditivos deste Comitê (11)

Em linhas gerais, para se estimar a ingestão provável de um aditivo são necessários dados de consumo ou previsão de consumo dos alimentos nos quais se pretende utilizar o aditivo e a concentração do aditivo nos alimentos. Se a ingestão provável decorrente de todas as fontes for inferior à IDA e houver justificativa tecnológica para o uso do aditivo, seu emprego em alimentos é aprovado.

A seguir são apresentados algumas informações relativas à avaliação toxicológica do corante natural urucum, bem como estimativa de sua ingestão no Brasil.

Avaliação toxicológica do urucum

Em 1982 foram avaliados pelo JECFA resultados de ensaios toxicológicos conduzidos com extratos de urucum, incluindo estudos sobre acúmulo e excreção, estudos de mutagênese, estudos de toxicidade aguda, estudos de curto prazo, estudos crônicos e observações no Homem (12). Com base em estudos crônicos em ratos expostos a extratos contendo 0,2-2,6% de carotenóides totais, expressos como bixina, foi calculado um NOEL de 0,5% (5000ppm) na dieta do rato, equivalentes a 250 mg/kg peso corpóreo. A ingestão diária aceitável do urucum para o Homem foi então extrapolada utilizando-se um fator de segurança de 100, conforme apresentado a seguir:

IDA = NOEL / FS = 250 x 2,6% /100
IDA = 0,065 mg/kg p.c. (expressa como bixina)

Ingestão potencial de bixina no Brasil

A partir de estudos conduzidos em 1998 no Estado da Bahia, Brasil, junto a grandes consumidores de urucum, foi estimada uma ingestão potencial de bixina/norbixina na faixa de 0,097 a 0,14 mg/kg pc (dados não publicados).

A estimativa de ingestão foi calculada a partir de dados de consumo de alimentos que contém urucum e dos níveis tecnológicos de uso, fornecidos pelos fabricantes. Entre os alimentos identificados na pesquisa, o condimento colorífico, largamente utilizado na culinária da Região Norte e Nordeste do Brasil, representou mais de 70% da ingestão estimada.

Embora o estudo tenha evidenciado uma ingestão de bixina acima do valor recomendado pelo JECFA (0,065 mg/kg pc), sabe-se que grandes quantidades de urucum fazem parte do hábito alimentar de algumas regiões do Brasil há mais de 100 anos, não havendo até o momento uma associação direta com qualquer tipo de doença.

MERCOSUL

No âmbito do MERCOSUL, os corantes naturais permitidos para uso em alimentos foram aprovados pela Resolução GMC no. 45/93 e seu emprego já foi harmonizado para oito categorias de alimentos. Os limites máximos aprovados variam conforme a informação disponível, havendo o compromisso por parte dos Estados Membros de reavaliar os niveis máximos propostos, após harmonizadas todas as categorias de alimentos, de forma a garantir que os usos aprovados para cada corante não resultarão em ingestão acima da respectiva IDA.

CONCLUSÃO

Em face às exigências crescentes por parte de órgãos de regulamentação, tem havido uma evasão gradual de corantes da lista permitida em muitos países, e é pouco provável que alguma indústria proceda à difícil tarefa de desenvolver novos corantes artificiais para alimentos, pelo menos nos moldes que vinham sendo utilizados. Embora haja uma tendência crescente ao uso de corantes naturais em alimentos, o fato de muitos destes corantes não terem sido avaliados quanto a sua segurança ou apresentarem valores de IDA relativamente baixos pode, no futuro, limitar sua utilização mais ampla pela indústria de alimentos. Torna-se, portanto, de extrema importância o desenvolvimento de especificações adequadas para os corantes naturais, de forma a possibilitar sua avaliação ou reavaliação toxicológica e, consequentemente, seu emprego mais amplo em alimentos, em conformidade com padrões internacionais de segurança.

REFERÊNCIAS

1. FAO/OMS. Codex Alimentarius Commission: Procedural manual. 10 ed. Rome, 1997.
2. WHO. Principles for the safety assessment of food additives and contaminants in food. Environmental Health Criteria Nº 70 Geneva: World Health Organization, 174pp, 1987.
3. Dawson RJ. The role of Codex Alimentarius Commission in setting food standards and the SPS agreement implementation. Food Control 1995; 1: 261-5.
4. Grupo Mercado Comum. Resolução GMC Nº 17/93, 1993
5. Kojima K. The toxicological assessment of natural food colorants. In: Galli CL, Paoletti R, Vettorazzi G, editors. Chemical toxicology of food. Elsevier/North Holland, 1978: 319-27.
6. Renwick AG. Safety factors and establishment of acceptable daily intakes. Food Addit Contam 1991; 8:135-50.
7. Truhaut R. The concept of acceptable daily intake: an historical review. Food Addit Contam 1991; 8:151-62.
8. Vettorazzi G. Advances in the safety evaluation of food additives. A conceptual and historical overview of the Acceptable Daily Intake (ADI) and Acceptable Daily Intake "not specified". Food Addit Contam 1987; 4: 331-56.
9. FAO-WHO-IPCS. Summary of evaluations performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Washington DC: ILSI Press, Rome: Food and Agricultural Organization of the United Nations, Geneva: World Health Organization, 1996.
10. CAC. Report of the 22nd Session of the Codex Alimentarius Commission, ALINORM 97/37, 1997.
11. CCFAC. Codex Committee on Food Additives and Contaminants, CX/FAC 99/4, 1999.
12. WHO. Toxicological evaluation of certain food additives and contaminants. WHO Food Additive Series 17. Geneva: World Health Organization, 1982.

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Urucum - avanços tecnológicos e perspectivas

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RESUMO. Os corantes derivados das sementes de urucum (*Bixa orellana,* L.) são extensivamente utilizados na indústria de alimentos. Suas características peculiares como a que possibilita a obtenção de corantes hidrossolúveis ou lipossolúveis a partir de pequenas alterações nos processos de produção, foi um dos fatores do sucesso deste pigmento. Observa-se que só recentemente, pouco mais de cinco anos, têm sido dedicados esforços no estudo das características físico-químicas das diferentes estruturas de carotenóides que participam do chamado corante de urucum. A própria indústria produtora de corante somente recentemente tem se atentado para a possibilidade de obtenção de corantes com tonalidades diferentes, a partir das sementes de urucum e dedicado estudos a este respeito. Por outro lado estão sendo cobrados estudos toxicológicos complementares sobre estes pigmentos fazendo com que sua utilização possa ser questionada e, somente com a conclusão destes trabalhos é que poderá avaliar o futuro deste corante.
Palavras chave: Urucum, corantes.

SUMMARY. Annatto: Technological advances and perspectives. Colorants derived from the seeds of annatto (*Bixa orellana,* L.) are extensively used in the food industry. Their pecular characteristics, as that which permits obtaining water-soluble and lipid-soluble colorants through small alterations of the production process, was one of the factors responsible for the success of this pigment. It can be observed that only recently, a little over five years, efforts have been dedicated to the study of the physico-chemical characteristics of the different carotenoid structures that compose the so-called annatto colorant. The very industry that produces the colorant has only recently perceived the possibility of obtaining colorants of different hues from annatto seeds and has dedicated studies to this respect. On the other hand, complementary toxicological studies of these pigments are being demanded, putting their utilization in question, and only with the conclusion of these work can their future be evaluated.
Key words: Annatto, colorants.

INTRODUÇÃO

Há pelo menos 60 anos atrás, já era publicado uma monografia sobre o urucueiro (*Bixa orellana,* L.) que o colocava como "uma cultura que embora relegada para o plano de completo indiferentismo, merece, contudo, o carinho de uma exploração em larga escala", prevendo que "este produto poderá vir a desempenhar um fator apreciável na balança econômica nacional". Estas previsões são confirmadas hoje, com os corantes derivados das sementes do urucum sendo extensivamente utilizados nas indústrias de alimentos. Suas características peculiares, que permitem a obtenção de corantes lipossolúveis ou hidrossolúveis a partir de pequenas alterações no processo de produção, assim como a utilização de tecnologias rudimentares para a obtenção do corante, tem sido um dos fatores de sucesso deste pigmento.

O estudo das características físico-químicas das diferentes estruturas dos carotenóides que participam do chamado "corante de urucum", tem sido objeto de vários trabalhos. Estes estudos podem permitir a obtenção de corantes com tonalidades diferentes, caracterizados pela participação diferenciada dos diversos pigmentos presentes.

Características dos corantes do urucum

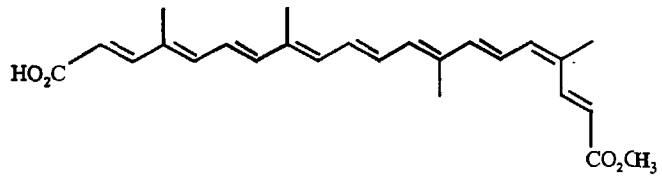
Algumas características peculiares do carotenóide presente em maior concentração nas sementes de urucum, a bixina, tem contribuído para o sucesso dos chamados "corantes de urucum".

A *cis*-bixina (Figura 1), que representa cerca de 80% dos pigmentos presentes na semente de urucum, é um éster monometílico de um ácido dicarboxílico. Esta estrutura, aliada às várias duplas ligações conjugadas de sua molécula, típica dos carotenóides, confere a este pigmento características interessantes como: a possibilidade de se obter corantes hidrossolúveis ou lipossolúveis a partir de pequenas alterações nos processos de produção, a propriedade de se ligar a determinadas proteínas, permanecendo no produto durante o processamento, e a característica de estar em uma das faixas de coloração mais utilizadas em alimentos (amarelo ao vermelho).

Os produtos de degradação térmica da bixina (carotenóides de menor peso molecular) são utilizados como pigmentos para alimentos que necessitem de uma coloração mais estável, como massas alimentícias que geralmente são comercializadas em embalagens com baixa barreira à luz.

A participação do urucum no mercado brasileiro se deve em grande parte à sua utilização na forma de colorífico. O colorpífico é o produto resultante da mistura do pigmento das sementes de urucum com farinha de milho ou de mandioca, óleo vegetal e sal e é utilizado como condimento e corante na culinária doméstica.

FIGURA 1



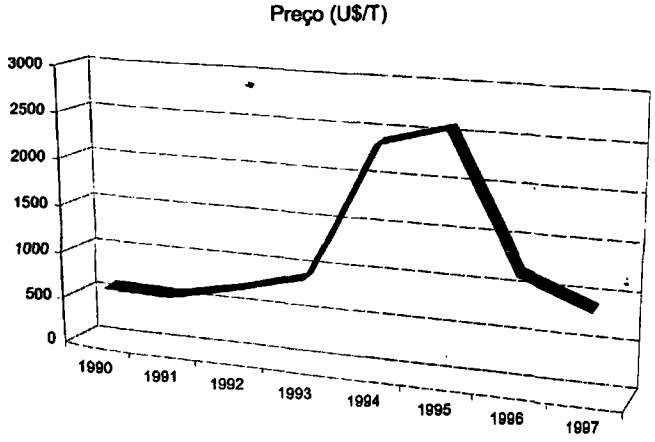
Mercado de sementes e "corantes de urucum"

Cerca de 60% do mercado brasileiro de sementes de urucum é representado pela produção e comércio do colorífico, cujo consumo está em torno de 500g anual, *per capita*, nas regiões norte e nordeste. Os outros 40% da produção nacional são distribuídos em aproximadamente 25% para a exportação de sementes *in natura* e 15% para a fabricação de corantes.

A produção brasileira é atualmente estimada em cerca de 7000 toneladas de sementes por ano, representando cerca de 40% da produção mundial. Os principais importadores de sementes de urucum do Brasil são os Estados Unidos, Inglaterra, França e Japão.

O preço das sementes de urucum passou por grandes variações nos anos de 93 a 96 (Figura 2), fazendo com que, se por um lado ocorresse uma diminuição da motivação dos produtores em anos de preço em baixa, por outro lado aumentasse o interesse pela cultura nos anos de preço elevado. Este aumento de interesse foi seguido pelo incremento dos estudos relativos aos aspectos agronômicos da cultura, gerando uma melhoria considerável na qualidade das sementes. Atualmente o mercado tem exigido um teor mínimo de 2,5% de bixina.

FIGURA 2
Variação de preço da semente de urucum no Brasil



Avanços tecnológicos e perspectivas

Os avanços tecnológicos relacionados aos "corantes de urucum" podem ser distribuídos quanto aos aspectos referentes à matéria prima, aos processos de produção e formulação dos corantes e sobre sua composição e características.

No que diz respeito à matéria prima, podemos afirmar que, tanto na produtividade como na qualidade das sementes, a melhoria conseguida a partir dos trabalhos desenvolvidos nos últimos anos foi muito significativa. O teor de bixina aumentou de uma média inferior a 2% para valores superiores a 2,5%. A produtividade média gira hoje em torno de 1500kg/ha a partir do 5° ano de produção.

As pesquisas com variedades mais produtivas e resistentes a pragas como o oídio, comum em área de alta umidade, e a "profissionalização" do cultivo do urucum, com investimentos adequados no manejo cultural, permitem prever uma melhoria na competitividade desta cultura, melhorando o ganho do produtor e estabelecendo preços compatíveis com as atividades das indústrias e corantes.

Quanto aos processos de produção do "corante de urucum" podemos separá-los em duas áreas. A primeira é representada pela produção do "corante pronto para o uso" onde se inclui o colorífico e todas as formulações voltadas ao uso direto pela indústria. Estes corantes são caracterizados pela concentração de carotenóides, inferior a 10%. A segunda área é representada pelos corantes na forma de pó, com concentrações superiores a 20%.

As tecnologias de produção dos corantes "pronto para o uso" não sofreram grandes alterações nos últimos anos, despontando como novidade o uso do próprio corante de urucum, pré-extraído com óleo vegetal, para a produção de colorífico, substituindo os processo tradicionais que utilizam extrações diretas das sementes.

Outra alteração observada e que deve ter progressos nos próximos anos é o estabelecimento de formulações com maior concentração do pigmento. Estas formulações visam fornecer para as indústrias, corantes com concentrações de bixina ou norbixina superiores a 5%, favorecendo o transporte e o armazenamento, sem aumentos proporcionais de preço.

As tecnologias voltadas para produção de corantes na forma de pó têm se caracterizado por produtos com concentrações inferiores a 50% de carotenóides, expressos em bixina. Isto está basicamente relacionado ao custo/benefício dos processos de purificação dos pigmentos extraídos.

Novas tecnologias têm sido estudadas, procurando conseguir, já no processo extrativo, corantes com elevadas concentrações de pigmentos. Entre estas técnicas destaca-se a extração com fluídos supercríticos.

Nos últimos anos tem sido dada maior atenção ao aspecto toxicológico dos pigmentos do urucum, visando estabelecer uma IDA (Ingestão Diária Aceitável) definitiva e com valores superiores à atualmente utilizada (0,065mg/kg p.c.). Esta preocupação é reforçada pela pressão exercida para a substituição dos "corantes de urucum" por outros corantes com menor IDA.

REFERÊNCIAS

1. Carvalho PRN, Yabiku HY. I Simpósio Internacional de Urucum. Campinas: ITAL, 1991.
2. Carvalho PRN, da Silva MG, Moreira CGC. Avaliação dos métodos espectrofotométricos de análise de sementes de urucum (*Bixa orellana,* L.). Colet ITAL 1993; 23: 181-8.
3. Carvalho PRN. Hein M. Urucum - Uma fonte de corante natural. Colet ITAL 1989; 19: 25-33.
4. da Silva GF. Extração de pigmentos do urucum com CO_2 supercrítico. Tese de doutorado. Campinas: FEA-UNICAMP, 1999.
5. do Canto WL, de Oliveira VP, Carvalho PRN, Germer SPM. Produção e mercado de urucum no Brasil. Estudos econômicos - Alimentos processados. Campinas: ITAL, 1991.

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Latin American food sources of carotenoids

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SUMMARY. Latin America has a wide variety of carotenogenic foods, notable for the diversity and high levels of carotenoids. A part of this natural wealth has been analyzed. Carrot, red palm oil and some cultivars of squash and pumpkin are sources of both ß-carotene and α-carotene. ß-carotene is the principal carotenoid of the palm fruits buritĺ, tucumã and bocaiuva, other fruits such as loquat, marolo and West Indian cherry, and sweet potato. Buriti also has high amounts of α-carotene and γ-carotene. ß-Cryptoxanthin is the major carotenoid in caja, nectarine, orange-fleshed papaya, orange, peach, tangerine and the tree tomato. Lycopene predominates in tomato, red-fleshed papaya, guava, pitanga and watermelon. Pitanga also has substantial amounts of ß-cryptoxanthin, γ-carotene and rubixanthin. Zeaxanthin, principal carotenoid of corn, is also predominant only in piquí. δ-Carotene is the main carotenoid of the peach palm and ζ-carotene of passion fruit. Lutein and ß-carotene, in high concentrations, are encountered in the numerous leafy vegetables of the region, as well as in other green vegetables and in some varieties of squash and pumpkin. Violaxanthin is the principal carotenoid of mango and mamey and is also found in appreciable amounts in green vegetables. Quantitative, in some cases also qualitative, differences exist among cultivars of the same food. Generally, carotenoids are in greater concentrations in the peel than in the pulp, increase considerably during ripening and are in higher levels in foods produced in hot places. Other Latin America indigenous carotenogenic foods must be investigated before they are supplanted by introduced crops, which are often poorer sources of carotenoids.
Key words: Carotenogenic foods, carotenoids, fruits, vegetables, root crops.

RESUMEN. Fuentes alimenticias latinoamericanas de carotenoides. América Latina posee una gran variedad de alimentos carotenogénicos, notables por la diversidad y altos niveles de carotenoides. Una parte de esta riqueza natural ya fue analizada. Zanahoria, aceite de palma roja y algunos cultivares de calabaza son fuentes tanto de ß-caroteno como de α-caroteno. ß-Caroteno es también el principal carotenoide de los frutos de palma buritĺ, tucumán y bocaiuva, otros frutos como néspera, marolo y acerola, y la patata dulce. La ß-criptoxantina es mayoritaria en cajá, nectarina, papaya anaranjada, naranja, melocotón, mandarina y tamarillo. El licopeno predomina en tomate, papaya roja, guayaba, pitanga y sandia. En la pitanga se encuentran también cantidades substanciales de ß-criptoxantina, γ-caroteno y rubixantina. La zeaxantina, principal carotenoide del maíz, es mayoritaria también solamente en piquí. El δ-caroteno es el carotenoide preponderante de la pupuña y el ζ-caroteno del maracujá. Luteina y ß-caroteno, en altas concentraciones, están presentes en las numerosas hojas de la región, como en otras verduras y ciertas variedades de calabaza. La violaxantina es el carotenoide predominante del mango y mamey, pero está también en verduras en cantidades apreciables. Existen diferencias cuantitativas y, a veces cualitativas, entre cultivares del mismo alimento. Generalmente, los carotenoides se encuentran en niveles mayores en la cáscara en vez de la pulpa, aumentan considerablemente durante la maduración y son mayores en alimentos producidos en los lugares más calientes. Otras fuentes alimenticias indígenas de la América Latina deben ser investigadas, antes que sean substituidos por cultivos introducidos, que son frecuentemente fuentes más pobres de carotenoides.
Palabras clave: Alimentos carotenogénicos, carotenoides, frutos, verduras, vegetales.

INTRODUCTION

With its tropical and sub-tropical areas, Latin America abounds in carotenogenic plant foods. Aside from internationally recognized food sources of carotenoids, there is a remarkable variety of lesser known or virtually unknown carotenoid-containing species. A part of this rich natural resource has been analyzed, particularly in Brazil. This Brazilian endeavor has generated one of the world's most extensive databases on food carotenoids, but because of the diversity of sources, there is an urgent need to extend analytical activities as the first step to better utilization of valuable but underexploited plant species. Because efforts along this line has been long in coming, a serious threat is the disappearance of carotenoid-rich indigenous species, supplanted by introduced crops, which are often poorer sources.

Fruits

The carotenoid composition of fruits is complex and variable (Table 1). Palm fruits, such as bocaiúva, buriti, tucumã and peach palm (pupunha), are rich in carotenes (i.e. hydrocarbon carotenoids). Buriti has the highest ß-carotene content of the foods already analyzed. Augmented by high levels of α-carotene and γ-carotene, which are also precursors

of vitamin A, this palm fruit presents a vitamin A value of about 6,500 RE (μg) /100 g. ß-Carotene also predominates in bocaiúva and tucumã, while the vitamin A-inactive δ-carotene prevails in peach palm, although it also contains good amounts of ß-carotene and γ-carotene. Bocaiúva comes from Mato Grosso do Sul and the other three palm fruits are harvested from wild trees, especially in Northern Brazil. Given their carotenoid content, their commercial production should be encouraged.

TABLE 1
Carotenoid composition (μg/g) of fruits

Fruit	Portion Analyzed	Origin	N	Carotenoids
Bocaiúva (*Acronomia makayáyba*)	pulp	MS	5	ß-carotene (59±11), γ-carotene (0.9±0.2), ß-cryptoxanthin (1.7±0.4), *cis*-lycopene (4.4±0.3), *cis*-flavoxanthin (0.2±0.1)
Buriti (*Mauritia vinifera* Mart)	pulp	PI	5	13-*cis*-α-carotene (1.5±1.4), α-carotene (80±9), 13-*cis*-ß-carotene (3.8±2.9), ß-carotene (360±32), 9-*cis*-ß-carotene (0.7±0.5), ζ-carotene (4.6±0.5), ß-zeacarotene (5.4±1.5), γ-carotene (36±4), zeaxanthin (20±4)
Cajá (*Spondias lutea*)	pulp + peel	PE	4	α-carotene (0.9±0.4), ß-carotene (1.6±0.2), ζ-carotene (0.3±0.1), zeinoxanthin (4.3±0.6), ß-cryptoxanthin (16±2), cryptoflavin (1.8±0.7), lutein (0.4±0.3)
	pulp	PE	1	α-carotene (2.1), ß-carotene (2.6), zeinoxanthin (1.7), ß-cryptoxanthin (8.3), cryptoflavin (0.6), lutein (2.0)
Cashew-apple (*Anacardium occidentale*) yellow type	whole fruit	SP,CE	2	α-carotene (0.1), ß-carotene (0.6), ζ-carotene (tr), *cis*-ß-carotene (0.3), ß-cryptoxanthin (0.5), aurochrome (tr), cryptochrome (tr), auroxanthin (tr)
red type	whole fruit	SP,PA	2	α-carotene (0.2), ß-carotene (1.7), ζ-carotene (tr), *cis*-ß-carotene (0.5), ß-cryptoxanthin (1.1), aurochrome (tr), cryptochrome (tr), auroxanthin (tr)
Guava (*Psidium guajava*) Cultivar IAC-4	whole fruit	SP	4	ß-carotene (3.7±0.7), ζ-carotene (tr), γ-carotene (tr), zeinoxanthin (1.0±0.6), lycopene (53±6), ß-carotene-5,6,5',6'-diepoxide (tr), trihydroxy-ß-carotene-5,8-epoxide (4.0±0.3)
	peeled fruit	SP	2	ß-carotene (5.0±1.2), ζ-carotene (tr), zeinoxanthin (0.5±0.1), lycopene (57±6), ß-carotene-5,6,5',6'-diepoxide (0.1±0.2), trihydroxy-ß-carotene-5,8-epoxide (2.0±0.2)
Undefined variety	whole fruit	PE	3	ß-carotene (12±5), γ-carotene (tr), *cis*-γ-carotene (tr), γ-carotene(0.4±0.3), zeinoxanthin (1.9±0.7), lycopene (53±14), ß-carotene-5,6,5',6'-diepoxide (0.1±0.1), zeinoxanthin-5,8-epoxide (0.2±0.1), trihydroxy-5,8-epoxy-ß-carotene (2.1±1.9)
Undefined variety	whole fruit	CE	2	ß-carotene (5.5±2.3), ζ-carotene (tr), γ-carotene (tr), zeinoxanthin (1.5±0.2), lycopene (47±16), ß-carotene-5,6,5',6'-diepoxide (0.3±0.4), trihydroxy-5,8-epoxy-ß-carotene (2.3±0.7)
Loquat (*Eriobotrya japonica* Lindl.) Mizuho	pulp	SP	5	ß-carotene (8.0±0.6), ζ-carotene (0.1±0.1), neurosporene (1.1±0.3), ß-cryptoxanthin (4.5±0.5), ß-cryptoxanthin-5,6-epoxide (0.6±0.2), violaxanthin (1.6±0.1), auroxanthin (0.9±0.1), neoxanthin (0.8±0.1)
Mamey (*Mammea americana*)	pulp	MA	13	13-*cis*-ß-carotene (0.3±0.2), ß-carotene (15±2), 9-*cis*-ß-carotene (0.2±0.2), ζ-carotene (1.1±0.6), ß-zeacarotene (0.5±0.3), ß-apo-10'-carotenal (4.8±0.8), ß-apo-8'-carotenol (12±7), violaxanthin (20±8), luteoxanthin (0.6±0.4), auroxanthin (1.8±0.6)
Mango (*Mangifera indica* L.) Keitt	ripe pulp	BA	3	ß-carotene (15±2), unidentified (0.2±0.0), *cis*-ß-cryptoxanthin (tr-0.1), ß-cryptoxanthin (0.3±0.0), zeaxanthin (0.8±0.2), luteoxanthin isomers (3.8±0.6), violaxanthin (21±3), 9-*cis*-violaxanthin (10±1), 13-*cis*-violaxanthin (1.4±0.1), *cis*-neoxanthin (tr-0.2), neoxanthin (2.1±1.3)
	mature-green pulp	SP	3	ß-carotene (1.7±0.3), unidentified (0.1±0.0), *cis*-ß-cryptoxanthin (tr), ß-cryptoxanthin (nd-tr), zeaxanthin (0.3±0.0), luteoxanthin isomers (1.0±0.2), violaxanthin (5.4±1.7), 9-*cis*-violaxanthin (1.7±0.4), 13-*cis*-violaxanthin (0.3±0.1), *cis*-neoxanthin (0.1±0.0), neoxanthin (1.6±0.6)
	ripe pulp	SP	3	ß-carotene (6.7±1.6), unidentified (0.2±0.0), *cis*-ß-cryptoxanthin (tr-0.1), ß-cryptoxanthin (0.2±0.0), zeaxanthin (0.8±0.3), luteoxanthin isomers (2.7±0.2), violaxanthin (18±4), 9-*cis*-violaxanthin (7.2±1.4), 13-*cis*-violaxanthin (nd-tr), *cis*-neoxanthin (0.3±0.2), neoxanthin (1.9±0.9)

Tommy Atkins	mature-green pulp	SP	3	ß-carotene (2.0±0.8), unidentified (nd-tr), *cis*-ß-cryptoxanthin (0.1±0.0), ß-cryptoxanthin (0.1±0.0), zeaxanthin (0.3±0.1), luteoxanthin isomers (1.3±0.7), violaxanthin (6.9±3.0), 9-*cis*-violaxanthin (3.3±1.3), 13-*cis*-violaxanthin (0.5±0.2), *cis*-neoxanthin (nd-tr), neoxanthin (2.6±1.8)
	ripe pulp	SP	3	ß-carotene (5.8±2.5), *cis*-ß-cryptoxanthin (0.1±0.1), ß-cryptoxanthin (0.3±0.1), zeaxanthin (0.4±0.2), luteoxanthin isomers (2.0±0.6), violaxanthin (22±9), 9-*cis*-violaxanthin (14±5), 13-*cis*-violaxanthin (tr), *cis*-neoxanthin (1.0±1.0), neoxanthin (4.9±4.5)
Marolo (*Annona coriaceae*)	pulp	MG	5	α-carotene (0.6±0.2), ß-carotene (7.0±4.0), ζ-carotene (0.1±0.0), ß-zeacarotene (0.1±0.0), ε-carotene (0.1±0.0), ß-cryptoxanthin (0.1±0.1), violaxanthin (0.1±0.1), lutein (0.7±0.2), mutatoxanthin (0.2±0.1)
Nectarine (*Prunus persica*)	pulp	SP	5	13-*cis*-ß-carotene (0.1±0.1), ß-carotene (1.0±0.2), 9-*cis*-ß-carotene (0.1±0.1), ζ-carotene (0.2±0.1), *cis*-ß-cryptoxanthin (0.3±0.2), ß-cryptoxanthin (3.9±0.7), lutein (1.1±0.2), zeaxanthin (1.6±0.3), violaxanthin (0.8±0.1), auroxanthin (0.4±0.3)
Papaya (*Carica papaya*) Common	pulp	SP	5	ß-carotene (1.2±0.9), ζ-carotene (0.8±0.5), ß-zeacarotene (0.1±0.0), ß-cryptoxanthin-5,6-epoxide (2.0±1.1), ß-cryptoxanthin (8.1±1.7), cryptoflavin (0.8±0.3)
Solo	pulp	BA	5	ß-carotene (2.5±1.0), ζ-carotene (1.4±0.8), γ-carotene (0.2±0.0), ß-cryptoxanthin-5,6-epoxide (tr), ß-cryptoxanthin (9.1±2.4), antheraxanthin (tr), lycopene (21±16)
Formosa	pulp	SP	5	ß-carotene (1.4±0.5), ζ-carotene (1.7±0.6), ß-cryptoxanthin-5,6-epoxide (3.8±1.3), ß-cryptoxanthin (5.3±1.1), antheraxanthin (1.8±0.1), lycopene (19±4)
Formosa	pulp	BA	5	ß-carotene (6.1±1.4), ζ-carotene (1.5±0.3), ß-cryptoxanthin-5,6-epoxide (1.8±0.8), ß-cryptoxanthin (8.6±2.2), antheraxanthin (3.3±0.4), lycopene (26±3)
Tailandia	pulp	BA	5	ß-carotene (2.3±0.7), ζ-carotene (2.0±0.4), ß-cryptoxanthin-5,6-epoxide (2.1±0.3), ß-cryptoxanthin (9.7±1.8), antheraxanthin (4.0±2.9), lycopene (40±6)
Peach (*Prunus persica*) Rei da Conserva	pulp	SP	3	13-*cis*-ß-carotene (0.2±0.1), ß-carotene (1.1±0.4), 9-*cis*-ß-carotene (0.1±0.0), ζ-carotene (0.4±0.2), *cis*-ß-cryptoxanthin (1.0±0.5), ß-cryptoxanthin (6.4±2.1), lutein (3.8±1.1), zeaxanthin (1.5±0.9), violaxanthin (0.8±0.6)
Diamante	pulp	SP	5	13-*cis*-ß-carotene (0.2±0.1), ß-carotene (0.6±0.2), 9-*cis*-ß-carotene (0.1±0.1), ζ-carotene (0.2±0.1), *cis*-ß-cryptoxanthin (0.2±0.1), ß-cryptoxanthin (4.1±0.8), violaxanthin (0.9±0.4), luteoxanthin (0.7±0.3), auroxanthin (0.2±0.2)
Chilean*	pulp		5	13-*cis*-ß-carotene (0.2±0.1), ß-carotene (1.2±0.2), 9-*cis*-ß-carotene (0.1±0.1), ζ-carotene (0.2±0.1), *cis*-ß-cryptoxanthin (0.3±0.2), ß-cryptoxanthin (5.1±1.5), lutein (2.2±0.4), zeaxanthin (0.2±0.2), violaxanthin (1.6±0.4), auroxanthin (0.8±0.4)
Peach palm (*Bactris gasipaes*)	boiled pulp	AM	5	α-carotene (3.2±3.1), ß-carotene (22±12), δ-carotene (25±9), γ-carotene (18±7)
Piqui (*Cariocar vilosium*)	pulp	PI	5	α-carotene (0.1±0.1), ß-carotene (1.2±0.5), ζ-carotene (0.5±0.3), *cis*-ß-cryptoxanthin (0.4±0.2), ß-cryptoxanthin (4.4±0.9), zeaxanthin (7.8±1.2)
Pitanga (*Eugenia uniflora*)	pulp	PE	18	phytofluene (13±2), ß-carotene (9.5±2.1), ζ-carotene (4.7±1.6), unidentified (3.4±0.4), ß-cryptoxanthin (47±2), γ-carotene (53±4), lycopene (73±1), rubixanthin (23±2)
Tree tomato (*Cyphomandra betacea*)	pulp	SP	5	ß-carotene (7.9±3.6), ζ-carotene (tr), ß-carotene-5,6-epoxide (0.3±0.1), ß-cryptoxanthin (14±4), lutein (1.7±1.1), zeaxanthin (0.6±0.6)
Tucumã (*Astrocaryum vulgare*)	pulp	AM	5	ß-carotene (107±31), ß-zeacarotene (5.9±3.1), ζ-carotene (6.2±2.2), γ-carotene (2.0±1.6)
West Indian Cherry (*Malpighia glabra*)	pulp	SP	4	α-carotene (tr), ß-carotene (4.0±0.6), ß-cryptoxanthin (0.5±0.2)
		PE	18	α-carotene (0.1±0.1), ß-carotene (26±4), ß-cryptoxanthin (3.6±0.7)
		CE	4	α-carotene (tr), ß-carotene (22±1), ß-cryptoxanthin (2.1±0.4)

Unless stated otherwise, the carotenoids are in *trans*-form and the samples are ripe. N - number of sample lots analyzed; MS - Mato Grosso do Sul, PI - Piauí, PE - Pernambuco, SP - São Paulo, CE- Ceará, PA - Pará, MA - Maranhão, BA - Bahia, MG Minas Gerais, AM - Amazonas, nd - not detected, tr - trace.
*Only non-Brazilian sample analyzed.
References: Hiani and Penteado (1), Godoy and Rodriguez-Amaya (2-4), Rodriguez-Amaya and Kimura (5), Cecchi and Rodriguez-Amaya (6), Padula and Rodriguez-Amaya (7), Rodriguez-Amaya et al. (8,9), Mercadante et al. (10), Mercadante and Rodriguez-Amaya (11), Agostini et al. (12), Kimura et al. (13), Godoy et al. (14), Cavalcante and Rodriguez-Amaya (15).

Since lipids are known to stimulate absorption of carotenoids, palm fruit carotenoids may have the added advantage of greater bioavailability. The peach palm is only eaten cooked, and in this form, the provitamins A were found to be highly bioavailable in rats, more bioavailable than those of fresh mango (16). Buriti sweet made from the pulp was shown to be well-accepted and effective in the prevention of hypovitaminose A and treatment of xerophthalmia in children of the semiarid Northeastern region of Brazil (17).

ß-carotene is also the main carotenoid of the non-palm fruits loquat, marolo and West Indian cherry (Table 1). Loquat and marolo are not rich sources of carotenoids, but loquat is available at the time of the year (May to October) when few carotenogenic fruits are available. West Indian cherry is a good source of ß-carotene and it is well known and appreciated for its very high vitamin C content. Data obtained with this fruit clearly showed climatic effect. The fruits coming from the neighboring hot Northeastern states of Pernambuco and Ceará presented similar composition, with carotenoid levels pronouncedly higher than those of fruits produced in the temperate state of São Paulo.

ß-Carotene has long been considered the predominating pigment of mango. However, a recent work (10,11) on Keitt and Tommy Atkins cultivars showed violaxanthin to be actually the major carotenoid (Table 1). Highly unstable, violaxanthin can be easily lost during analysis, and this probably led to its underestimation in earlier studies.

Mercadante and Rodriguez-Amaya (11) also demonstrated the increase in carotenogenesis, especially of the major carotenoids, in both Keitt and Tommy Atkins cultivars, as the fruits ripened from the mature-green stage (Table 1). This is an important point to consider since mangoes are consumed at the green stage (usually more immature than the mangoes analyzed in the study) in some countries. Moreover, climatic effects were evident with the Keitt mangoes, those produced in the hot Northeastern state of Bahia presenting distinctly higher carotenoid levels than those from São Paulo.

Mango is produced in considerable amounts in Latin America. Unfortunately, it is seasonal and the peak harvest coincides with that of many carotenogenic fruits.

Violaxanthin is also the main carotenoid of mamey. This fruit is notable for having appreciable amounts of an apocarotenal and an apocarotenol (Table 1).

The xanthophyll (i.e. oxygenated carotenoid) ß-cryptoxanthin is the principal carotenoid of cajá, nectarine, orange-fleshed papaya, peach and the tree tomato. Peach and nectarine, together with apricot and plums, are apparently the only fruits produced in colder regions which contain appreciable amounts of carotenoids. The anthocyanin-colored fruits, such as apple, pear, prunes and grapes, are very low in carotenoids. Belonging to the same family, peach and nectarine have similar carotenoid composition (Table 1).

The data on cajá (Table 1) show that the carotenoids are more concentrated in the peel than in the pulp, as in most fruits (18,19). Peeling cajá not only reduce the carotenoid concentration but also the amount of available edible material (8 g/fruit vs. 2g/fruit). Thus, peeling is not recommended when the peel is edible. An exception to this common pattern is the pink-fleshed guava in which ß-carotene appeared slightly higher in the peeled fruit (Table 1) and lycopene is concentrated in the pulp.

Although native to Brazil, the tree tomato is hardly encountered in this country. It is better known and consumed in Ecuador. It is also found in Asian (e.g. New Zealand) and African (e.g. South Africa) countries.

Tangerine, orange and persimmons would also contain ß-cryptoxanthin as the main carotenoids. Citrus fruits are being currently analyzed (20), a study which is long overdue, considering that Brazil is one of the world's major producers of oranges and production of this fruit far exceeds that of other fruits. Although a good part is exported, domestic consumption of orange is substantial.

The vitamin A-inactive but efficient antioxidant lycopene is the preponderant carotenoid of pink-fleshed guava, red-fleshed papaya and pitanga (Table 1). Aside from being a rich source of lycopene, guava is also high in vitamin C.

Papaya is available all year round at prices the population can afford and enjoys wide acceptability by both children and adults. Aside from qualitative and quantitative variation in the other carotenoids, cultivar differences is notable in lycopene, from not detected in the orange-fleshed common cultivar to a level twice as much in the Tailandia papaya, compared to the other red-fleshed papayas (Table 1).

Climatic or geographic effect is also demonstrated in the Formosa papaya. As compared to those from São Paulo, Formosa papaya from Bahia had higher ß-carotene, ß-cryptoxanthin and lycopene contents (Table 1).

Pitanga has an interesting carotenoid composition, with high levels of lycopene, rubixanthin (monohydroxy derivative of γ-carotene), ß-cryptoxanthin and γ-carotene (Table 1). This fruit and West Indian cherry, both from Northeastern Brazil, have now been transformed from semi-cultivated to commercially produced fruits, with commercial production still expanding.

Piqui was considered for a long time as a very rich source of provitamin A. Analytical data did not confirm this belief. Piqui is low in carotenoids and the principal pigment is zeaxanthin (Table 1), which is vitamin A-inactive but is one of the two carotenoids implicated in the prevention of macular degeneration.

Cashew-apple is a poor source of carotenoids, but the cashew-apple juice is manufactured from the pseudofruit, a by-product of the cashew nut industry, thus turning what would otherwise be a waste product into a nutritious, aromatic juice. The vitamin C content is claimed to be several times higher than that of orange juice.

Passion fruit is also native to Brazil, and is now widely produced throughout the tropics. Brazilian production of this

fruit surpasses that of mango, guava and papaya; Brazil is the leading exporter of passion fruit juice. The carotenoid composition of this fruit is considered unusual because ζ-carotene is the principal carotenoid (21). The quantitative composition was not determined.

Other carotenogenic fruits, which are commercially produced and should be analyzed, are avocado, banana, melon, watermelon and pineapple. Banana is a poor source of carotenoids, but the amount of this fruit consumed by the population may increase its importance. It is, for example, the principal source of carotenoids in the Panamanian diet (22). There are still many indigenous fruits that await analyses.

Leafy vegetables

In contrast to fruits, leaves have a constant qualitative carotenoid pattern, the major carotenoids being lutein, ß-carotene, violaxanthin and neoxanthin. α-Carotene, ß - or α-cryptoxanthin, zeinoxanthin, antheraxanthin and lutein-5,6-epoxide can be encountered as minor constituents. Appreciable amount of lactucaxanthin is found in lettuce (23). Considerable quantitative differences occur among leaves.

The bioavailability of carotenoids from leaves is known to be lower than that of fruit carotenoids. On the other hand, the carotenoid contents of the former usually surpass those of the latter. Moreover, leaves are available all year round, easily produced in home gardens and are the most widely available and affordable sources of carotenoids worldwide.

Latin America has an enormous variety of wild, semi-cultivated and commercially produced leafy vegetables. As in other developing regions of the world, the tropical climate promotes the growth of green leafy spontaneous plants such as *Anaranthus spp, Hibiscus spp.* and *Basella spp.*, which, in many cases, have higher overall food value than introduced vegetable species.

In Campinas, São Paulo, the ß-carotene concentrations of common commercial leaves were determined in 5 to 15 sample lots for each leaf collected at different times during the year (24). The results in μg/g were: parsley (*Petroselium hortense*), 50±15; roquette (*Eruca sativa*), 35±13; coriander leaves (*Coriandrum sativum*), 47±5; cress (*Nastrutium officinale*), 42±10; kale (*Brassica oleracea* var. *acephala*), 35±13; common chicory (*Chicorium intybus*), 34±10; endive (*Chicorium endivia*), 17±6; curly, unheaded lettuce (*Lactuca sativa*), 14±5; Boston lettuce, 13±5; cabbage (*Brassica oleracea* var. *capitata*), 0.8±0.7; Chinese cabbage (*Brassica chinensis*), 1.0±1.4. Although the ß-carotene values were reliable, those of lutein, which were also determined, were considerably underestimated in this study because of the saponification step. This work was undertaken before saponification was evaluated in detail (25). Thirteen sample lots each of mustard leaves (*Brassica juncea*), Swiss chard (*Beta vulgaris*) and taioba (*Xanthosoma spp.*) from the city of São Paulo were also analyzed and found to have ß-carotene levels of 60±15, 13±11 and 66±14 μg/g, respectively (26). New Zealand spinach (Spinacea oleracea) (5 sample lots) had 25±4 μg/g of *trans*-ß-carotene (27).

The ß-carotene concentrations of the mature leaves of lettuce (12 μg/g) and endive (14 μg/g) were 3 times greater than those of the young leaves (3.5 and 4.2 μg, respectively), taken from the same bunches of leafy vegetables (24). The internal leaves had 0.38±0.01 μg/g ß-carotene as compared to 16±2 μg/g in external leaves in 10 samples of cabbage (28).

The ß-carotene contents of edible leaves from the state of Pará, Northern Brazil were also determined, one sample lot being analyzed in December and, for some samples, another sample lot in May (29). For endive, one sample lot was analyzed in May. The ß-carotene levels (μg/g) were: beldroega (*Portulaca holimoides*), 27; bertalha (*Basella rubra*), 55; cariru (*Talinum sp.*), 12 and 30; endive (*Chicorium intybus*), 25; Swiss chard (*Beta vulgaris*), 49; African spinach (*Amaranthus sp.*), 39; Indian spinach (*Amaranthus sp.*), 47 and 79; jambu branco (*Spilanthes acmella*), 39; cassava (*Manihot esculenta*), 151 and 108; mentruz (*Chenopodium ambrosioides*), 49 and 60; orelha de macaco (*Alternanthera sp.*), 33 and 59; tomato (*Lycopersicum esculentum*), 55; vinagreria branca (*Hibiscus sabdariffa*), 71 and 102; vinagreria roxa (*Hibiscus acetosila*), 73 and 84. These leaves have comparatively higher ß-carotene concentrations than the internationally known, commercially produced leafy vegetables in São Paulo.

The only published Latin American paper which presented quantitative data of the principal carotenoids of leaves is shown in Table 2, referring to five wild or semi-cultivated indigenous leaves. Except for beldroega, these leaves had higher ß-carotene levels than parsley, which presented the highest ß-carotene content among the commercially produced leaves of Campinas. Even beldroega, which had the lowest ß-carotene level among the native leaves, surpassed four of the commercial vegetables. Beldroega is more widely consumed in Mexico than in Brazil.

Cultivar difference, seasonal variation and effect of farming practice on the carotenoid composition were studied in mature kale leaves taken from commercial farms (31). Carotenoid levels were higher in the cultivar Tronchuda in the summer, but no statistically significant difference was seen between Tronchuda and Manteiga cultivars in the winter. The ß-carotene, lutein-violaxanthin and total carotenoid contents were higher in the winter than in the summer for the cultivar Manteiga. On the other hand, neoxanthin was higher in the summer for the Tronchuda kale. All constituent carotenoids were higher in samples from a "natural" farm as compared to those from a neighboring farm that used agrochemicals.

The edible leaves of carrots, which would usually be discarded, was found to have 2.1±1.0 μg/g of α-carotene and 27±11 μg/g of ß-carotene in the cultivar Brasília and 8.0±5.4 μg/g of α-carotene and 20±9 μg/g of ß-carotene in the cultivar Beta 3 (32).

TABLE 2
Carotenoid composition (μg/g)* of native leafy vegetables

Vegetable	ß-Carotene	α-Cryptoxanthin	Lutein + violaxanthin	Zeaxanthin	Neoxanthin
Beldroega (*Portulaca oleracea*)	30±8	0.6±0.8	48±8	0.7±1.5	9±2
Caruru (*Amaranthus viridis*)	110±6	1.3±1.2	237±50	8.2±6.5	43±5
Mentruz (*Lepidium pseudodidymum*)	85±19	nd	164±32	1.0±2.1	36±6
Serralha (*Sonchus oleraceus*)	63±14	0.3±0.6	145±52	3.1±5.7	29±6
Taiobá (*Xanthosoma spp.*)	67±21	1.0±1.4	172±38	2.7±6.0	40±10

*Means and standard deviations of five sample lots collected at different times during the year, nd - not detected.
Reference: Mercadante and Rodriguez-Amaya (30).

A very promising leaf is the chaya (*Cnidoscolus aconitifolius*), which is native to Mexico and Central America. It is consumed boiled to eliminate toxic substances. The shrub requires very little care and produces a large quantity of leaves for many years. The chaya leaf has been the object of investigations in Guatemala and was found to have 150 μg/g of ß-carotene, higher than in many other leaves (33).

Fruit vegetables

Squashes and pumpkins are easy to produce, widely available all year round and are rich in carotenoids. Many different varieties of these fruit vegetable can be found in Latin America and the rest of the world.

Aside from varietal differences, substantial variations in the carotenoid concentrations of the same variety of squash or pumpkin can be noted in Table 3. The magnitude of variation is so much greater than that observed in other fruits and vegetables that the ranges, rather the means and standard deviations, are presented in the table. This can be attributed to the long period during which the Cucurbita fruits can be harvested at varying degrees of maturity. Also, while other fruits and vegetables need to be consumed within a limited period after harvest, intact squashes and pumpkins have a very long shelf-life, during which the biochemical processes continue. No attempt was made to choose samples according to exact maturity stages or time after harvest so as to reflect the type of variation consumers would be exposed to.

ß-Carotene predominates in *C. moschata* Menina Verde (mature) and Baianinha and lutein in C. *maxima* Jerimum Caboclo. *C. maxima* Exposição and the hybrid Tetsukabuto have nearly the same amounts of these two carotenoids.

Cucurbita moschata Baianinha from Northeastern Brazil is notably rich in carotenoids. The hybrid Tetsukabuto, which is grown from imported seeds, has about the same carotenoid content as *C. maxima* Exposição, lower than those of the other three *Curcubita* fruits. Many other *Curcubita* varieties in Brazil and other Latin American countries have not been analyzed.

Red, green, yellow and orange varieties of pepper are marketed in Latin America. In Mexico, where consumption of pepper is so much a part of tradition and culture, the provitamin A carotenoids of five cultivars (Verde, Serrano, Jalapeño, Poblano and Caribe) of immature peppers were determined (38). ß-Carotene ranged from 1.7 to 6.0 μg/g, α-carotene from 0.17 to 1.1 μg/g, and ß-cryptoxanthin from not detected to 0.07 μg/g.

In yellow pepper from São Paulo, lutein is the principal carotenoid of Zarco Hybrid F_1 and Sunboy Hybrid F_1 while ß-cryptoxanthin-5,6,5',6'-diepoxide predominates in Amador Hybrid F_1 (Table 3).

Different varieties of tomato, the universal rich source of lycopene, is found throughout Latin America, although other sources of lycopene are also available. Guava, papaya cultivar Tailandia and pitanga (Table 1) were found to have higher lycopene content than the common Brazilian Santa Cruz tomato (Table 3). Other cultivars of tomato may have higher lycopene concentration.

Other non-leafy vegetables were analyzed for their provitamin A content (27). Except for broccoli flowerlets (ß-carotene, 18±1), the other vegetables such as green beans, okra and Indian eggplant had low vitamin A value.

Root crops

Carotenes, particularly α- and ß-carotene, generally predominate in the few carotenoid-containing roots. α-Carotene and ß-carotene account for 80 to 90% of the total carotenoid content of carrot (*Daucus carota*). Carrot cultivars Nantes and Imperador produced in São Paulo have about 20 μg/g of α-carotene and 35 μg/g of ß-carotene (27,39).

Cassava (*Manihot esculenta*) is a very popular food in Brazil and other Latin American countries. The cassava leaf is

TABLE 3
Carotenoid composition (μg/g) of fruit vegetables

Vegetable	Portion Analyzed	Origin	N	Carotenoids
Squash and Pumpkin (*Cucurbita maxima*) Exposição	pulp	SP	5	α-carotene (nd-0.2), ß-carotene (3.1-28), *cis*-ζ-carotene (nd-0.6), mutatochrome (nd-0.4), α-cryptoxanthin (nd-3.5), ß-cryptoxanthin (nd-0.8), cryptoflavin (nd-0.1), lutein (7.2-25), *cis*-lutein (nd-9.7), zeaxanthin (nd-9.7), taraxanthin (nd-3.6), violaxanthin (nd-26), *cis*-luteoxanthin (nd-0.9), trihidroxy-α-carotene (nd-1.0), neoxanthin (nd-4.2)
Jerimum Caboclo	pulp	PE	3	α-carotene (0.2-0.6), ß-carotene (14-34), *cis*-ß-carotene (1.5-2.7), α-cryptoxanthin (tr-6.7), α-cryptoxanthin-5,6-epoxide (nd-8.8), lutein (6.4-129), *cis*-lutein (nd-0.4), zeaxanthin (nd-0.2), taraxanthin (nd-6.0), *cis*-flavoxanthin (nd-6.0)
(*Cucurbita moschata*) Menina Verde	immature fruit	SP	5	α-carotene (tr-0.2), ß-carotene (0.8-2.5), *cis*-ß-carotene (nd-tr), mutatochrome (nd-0.1), α-cryptoxanthin (tr-0.5), α-cryptoxanthin-5,6-epoxide (nd-tr), lutein (0.7-7.4), *cis*-lutein (nd-0.4), taraxanthin (nd-1.2), violaxanthin (nd-0.3), *cis*-violaxanthin (nd-0.4), *cis*-antheraxanthin (nd-0.2), *cis*-luteoxanthin (nd-tr), neoxanthin (nd-tr)
	mature fruit	SP	5	α-carotene (8.3-42), ß-carotene (14-79), *cis*-ζ-carotene (0.9-20), α-zeacarotene (nd-13), mutatochrome (nd-2.1), aurochrome (nd-0.3), δ-carotene (nd-0.6), γ-carotene (nd-tr), α-cryptoxanthin (tr-2.3), lutein (tr-6.4), *cis*-lutein (0.2-3.1), violaxanthin (nd-3.3), *cis*-violaxanthin (nd-2.4), cis-luteoxanthin (nd-tr)
Baianinha	pulp	BA	3	α-carotene (17-82), ß-carotene (125-294), ß-carotene-5,6-epoxide (nd-2.2), *cis*-ß-carotene (4.9-30), α-zeacarotene (nd-1.7), neuroposrene (nd-tr), mutatochrome (nd-tr), δ-carotene (tr-0.7), α-cryptoxanthin (2.2-2.8), zeinoxanthin (tr-6.3), *cis*-ß-cryptoxanthin-5,6,5',8'-diepoxide (nd-0.3), lutein (4.8-14), taraxanthin (nd-tr), *cis*-flavoxanthin (nd-0.7), *cis*-violaxanthin (tr-0.9), luteoxanthin (nd-0.9), *cis*-luteoxanthin (nd-0.5), auroxanthin (nd-0.3)
Hybrid Tetsukabuto	pulp	BA	3	α-carotene (nd-0.5), ß-carotene (8.7-18), *cis*-ß-carotene (nd-0.1), α-zeacarotene (nd-0.2), neuroposrene (nd-5.4), mutatochrome (nd-0.3), zeinoxanthin (0.6-10), ß-cryptoxanthin (0.8-18), lutein (3.5-34), *cis*-lutein (nd-0.5), zeaxanthin (tr-6.5), taraxanthin (nd-8.5), flavoxanthin (nd-0.3), *cis*-violaxanthin (tr-2.7), luteoxanthin (nd-0.6), trihidroxy-α-carotene (nd-0.4), neoxanthin (nd-0.9)
Pepper (yellow) (*Capsicum annuum*) Amador Hybrid F_1	fruit	SP	1*	α-carotene (1.3), ß-carotene (2.2), *cis*-ζ-carotene (tr), unidentified (1.1), ß-carotene-5,6,5',6'-diepoxide (3.9), ß-cryptoxanthin-5,6,5',6'-diepoxide (12), ß-cryptoxanthin (0.2), lutein (3.9), violaxanthin (4.0)
Zarco Hybrid F_1	fruit	SP	1*	α-carotene (0.9), ß-carotene (1.1), *cis*-ζ-carotene (tr), unidentified (1.0), ß-carotene-5,6,5',6'-diepoxide (1.6), ß-cryptoxanthin-5,6,5',6'-diepoxide (3.4), ß-cryptoxanthin (tr), lutein (5.5), violaxanthin (2.2)
Sunboy Hybrid F_1	fruit	SP	1*	α-carotene (0.5), ß-carotene (1.2), cis-ζ-carotene (tr), unidentified (0.6), ß-carotene-5,6,5',6'-diepoxide (1.6), ß-cryptoxanthin-5,6,5',6'-diepoxide (2.5), ß-cryptoxanthin (tr), lutein (4.1), violaxanthin (1.5)
Tomato (*Lycopersicon esculentum*) Santa Cruz	fruit	SP	10	*cis*-phytofluene (3.7±4.6), ß-carotene (5.1±1.1), ζ-carotene (0.4±0.2), γ-carotene (0.7±0.2), *cis*-lycopene (3.0±2.4), lycopene (31±20)

Unless stated otherwise, the carotenoids are in *trans*-form and the samples are ripe.
N - number of sample lots analyzed; SP - São Paulo, PE - Pernambuco, BA - Bahia, nd - not detected, tr - trace.
*Values are means of 5 determinations of samples taken from the same sample lot.
References: Arima and Rodriguez-Amaya (34, 35), Bianchini and Penteado (36), Tavares and Rodriguez-Amaya (37).

a rich source of ß-carotene and lutein. The root, however, is low in carotenoids. Five cultivars of cassava (IAC 576-70, Ouro do Vale, Pioneira, IAC289-70, Branca de Santa Catarina) produced in São Paulo had 0.1 to 0.6 μg/g of *trans*-ß-carotene (40).

Mandioquinha (*Arracacia xanthorrhize*) also from São Paulo, had traces of α-carotene and 0.8±0.2 μg/g of ß-carotene (41).

Sweet potatoes, especially the yellow and orange varieties, are important sources of ß-carotene worldwide. The leaves are also consumed and should be good sources of ß-carotene and lutein. In Brazil, however, sweet potato is not so popular and the varieties cultivated (usually white varieties) are not high in provitamin A. Table 4 shows the wide variation in the ß-carotene content (not detected to 218 μg/g) of sweet potato cultivars. The American cultivars far surpassed the Brazilian varieties. Other Latin American countries cultivate colored varieties.

TABLE 4
Carotenoid composition (μg/g)* of sweet potatoes produced in Brazil

Cultivar	ß-Carotene	ß-Carotene -5,6-epoxide	ß-Carotene 5,6,5',6'-epoxide	Luteochrome	α-Zeacarotene	ß-Zeacarotene	Aurochrome
Monalisa	0.4±0.2	0.2±0.0	0.6±0.1	1.6±0.2	0.5±0.2	——	0.9±0.2
Centennial	149±1	8.3±0.0	4.6±0.5	1.5±0.7	——	3.4±0.7	2.6±0.1
Clone CNPH	4.9±0.2	0.6±0.3	1.2±0.7	2.9±0.4	0.6±0.1	——	——
Heart Gold	52±10	2.6±0.1	2.0±0.5	2.4±0.3	——	2.1±0.5	1.5±0.4
Anápolis	14±6	0.6±0.0	0.3±0.1	0.8±0.2	——	0.7±0.0	0.7±0.1
Acadian	218±34	5.8±1.7	3.0±1.5	4.9±0.1	——	2.2±0.2	1.7±0.1
Morada Inta	11±4	0.3±0.1	0.2±0.1	0.6±0.3	0.5±0.1	——	——
IAC-2-71	——	0.1±0.0	0.2±0.1	0.8±0.2	0.2±0.0	——	0.7±0.1
SRT-252	0.1±0.1	0.2±0.0	0.4±0.0	0.9±0.1	0.3±0.1	——	0.8±0.0
Vineland Bush	23±2	2.0±0.5	2.7±0.5	2.4±0.2	0.9±0.1	——	2.1±0.2

*Values are means and standard deviations of three determinations of samples taken from the same sample lot.
Reference: Almeida-Muradian and Penteado (42).

Processed foods

To utilize seasonal crops efficiently at peak harvest, permit wider distribution of products and provide a year-round supply, food processing is undertaken. The major part of the tomato crop, for example, is transformed into tomato puree, paste, ketchup and juice. Processed foods constitute a good part of the Latin American food market, and some of these foods had been analyzed (Table 5).

TABLE 5
Carotenoid composition (μg/g) of commercial processed foods

Product	Origin	N	Carotenoids
Cashew-apple juice, bottled			
Brand A	PE	3	α-carotene (tr), ß-carotene (0.7±0.1), ζ-carotene (tr), *cis*-ß-carotene (0.1±0.1), ß-cryptoxanthin (0.5±0.0), aurochrome+cryptochrome+auroxanthin (0.6±0.1)
Brand B	CE	3	α-carotene (tr), ß-carotene (0.7±0.1), ζ-carotene (tr), *cis*-ß-carotene (0.1±0.0), ß-cryptoxanthin (0.5±0.1), aurochrome+cryptochrome+auroxanthin (0.5±0.1)
Corn, canned	SP	3	13-*cis*-ß-carotene (0.3±0.2), ß-carotene (0.9±0.2), 9-*cis*-ß-carotene (0.4±0.2), ζ-carotene (0.2±0.1), *cis*-ß-cryptoxanthin (0.3±0.1), ß-cryptoxanthin (1.9±0.5), lutein (1.6±1.4), zeaxanthin (9.8±2.1), mutatoxanthin (1.1±0.4)
Mango juice, bottled			
Brand A	SP	3	ß-carotene (7.8±0.9), unidentified (0.1±0.0), ß-cryptoxanthin (0.1±0.0), auroxanthin (3.8±1.1)
Brand B	SP	3	ß-carotene (12±1), ß-cryptoxanthin (0.3±0.0), auroxanthin (5.8±0.2)
Brand C	SP	3	ß-carotene (6.3±1.3), ß-cryptoxanthin (0.2±0.1), auroxanthin (6.4±4.3)
Passionfruit juice, bottled	PE	3	α-carotene (tr), ß-carotene (1.1±0.1), ζ-carotene (3.6±0.8), *cis*-neurosporene (0.4±0.0), neurosporene (1.1±0.0), γ-carotene (tr), lycopene (tr), aurochrome (tr), cryptochrome (tr), auroxanthin (tr)
	CE	3	α-carotene (tr), ß-carotene (2.3±0.0), ζ-carotene (6.3±0.4), *cis*-neurosporene (0.5±0.1), neurosporene (2.2±0.1), γ-carotene (tr), aurochrome (tr), cryptochrome (tr), auroxanthin (tr)
Peach, canned	SP	3	13-*cis*-ß-carotene (0.2±0.0), ß-carotene (1.0±0.3), 9-*cis*-ß-carotene (0.2±0.1), ζ-carotene (0.1±0.1), *cis*-ß-cryptoxanthin (0.5±0.1), ß-cryptoxanthin (2.5±0.3), lutein (0.6±0.6), zeaxanthin (0.8±0.3), luteoxanthin (0.3±0.1)
Peach juice, bottled	SP	3	13-*cis*-ß-carotene (0.2±0.0), ß-carotene (0.9±0.2), 9-*cis*-ß-carotene (0.2±0.1), ζ-carotene (0.2±0.1), *cis*-ß-cryptoxanthin (0.3±0.1), ß-cryptoxanthin (1.6±0.4), lutein (1.3±0.3), zeaxanthin (0.4±0.1), auroxanthin (0.8±0.5)
Tomato juice, bottled			
Brand A	SP	3	*cis*-phytofluene (5.1±1.4), 13-*cis*-ß-carotene (0.02±0.01), ß-carotene (2.0±0.5), ζ-carotene (1.3±0.3), *cis*-lycopene (7.1+5.5), *trans*-lycopene (62±8)
Tomato puree			
Brand A, cartoned	SP	3	*cis*-phytofluene (9.4±0.9), 13-*cis*-ß-carotene (1.1±1.0), ß-carotene (3.0±0.9), 9-*cis*-ß-carotene (0.3±0.2), ζ-carotene (3.3±1.5), *cis*-lycopene (16±9), lycopene (133±8)
Brand A, bottled	SP	3	*cis*-phytofluene (14±1), 13-*cis*-ß-carotene (1.0±0.5), ß-carotene (4.3±1.4), ζ-carotene (2.2±0.2), *cis*-lycopene (14±12), lycopene (134±58)

Brand A, canned	SP	3	*cis*-phytofluene (14±9), 13-*cis*-ß-carotene (2.1±1.5), ß-carotene (4.4±2.5), 9-*cis*-ß-carotene (0.5±0.4), ζ-carotene (3.2±2.7), *cis*-lycopene (5.6±2.4), lycopene (114±89)
Brand B, cartoned	SP	3	*cis*-phytofluene (11±1), 13-*cis*-ß-carotene (1.8±0.7), ß-carotene (5.0±1.1), 9-*cis*-ß-carotene (0.6±0.1), ζ-carotene (2.6±1.1), *cis*-lycopene (3.6±2.0), lycopene (88±43)
Brand B, bottled	SP	3	*cis*-phytofluene (12±2), 13-*cis*-ß-carotene (1.3±1.1), ß-carotene (6.2±1.4), 9-*cis*-ß-carotene (0.4±0.2), ζ-carotene (3.3±0.7), *cis*-lycopene (5.6±1.3), lycopene (194±81)
Brand B, canned	SP	3	*cis*-phytofluene (11±4), 13-*cis*-ß-carotene (1.2±0.1), ß-carotene (3.9±1.0), 9-*cis*-ß-carotene (0.5±0.1), ζ-carotene (1.4±0.2), *cis*-lycopene (18±4), lycopene (74±18)
Tomato paste			
Brand A, bottled	SP	3	*cis*-phytofluene (10±3), 13-*cis*-ß-carotene (1.9±0.9), ß-carotene (8.7±3.2), 9-*cis*-ß-carotene (0.2±0.3), ζ-carotene (4.3±1.4), *cis*-lycopene (31±22), lycopene (170±61)
Brand A, canned	SP	3	*cis*-phytofluene (17±2), 13-*cis*-ß-carotene (2.0±1.8), ß-carotene (6.6±1.3), ζ-carotene (5.0±1.1), *cis*-lycopene (21±8), lycopene (164±53)
Brand B, bottled	SP	3	*cis*-phytofluene (9.2±2.8), 13-*cis*-ß-carotene (1.2±0.3), ß-carotene (5.9±1.0), 9-*cis*-ß-carotene (0.5±0.2), ζ-carotene (2.5±0.7), *cis*-lycopene (8.3±3.4), lycopene (158±22)
Brand B, canned	SP	3	*cis*-phytofluene (13±4), 13-*cis*-ß-carotene (2.6±0.3), ß-carotene (4.3±0.8), 9-*cis*-ß-carotene (1.0±0.1), ζ-carotene (2.7±0.1), *cis*-lycopene (15±6), lycopene (183±23)
Ketchup			
Brand A	SP	3	*cis*-phytofluene (16±1), 13-*cis*-ß-carotene (0.3±0.2), ß-carotene (3.5±0.8), 9-*cis*-ß-carotene (0.7±0.1), ζ-carotene (3.6±0.2), *cis*-lycopene (10±2), lycopene (103±41)
Brand B	SP	3	*cis*-phytofluene (8.5±0.6), 13-*cis*-ß-carotene (0.5±0.1), ß-carotene (3.5±0.6), 9-*cis*-ß-carotene (0.5±0.1), ζ-carotene (1.5±0.6), *cis*-lycopene (6.3±5.6), lycopene (86±30)

Unless stated otherwise, the carotenoids are in *trans*-form. N - number of sample lots analyzed; PE - Pernambuco, CE- Ceará, SP - São Paulo, tr - trace. References: Cecchi and Rodriguez-Amaya (6, 43), Tavares and Rodriguez-Amaya (37, 44), Mercadante and Rodriguez-Amaya (11).

The carotenoid composition of processed foods would depend on the composition of the raw material and the processing conditions, which could lead to varying degrees of degradation of the unstable carotenoids. Thus, the carotenoid levels of processed foods are often lower than those of the raw materials, unless concentration is part of the process as in the manufacture of tomato puree and paste. For better retention of carotenoids, effective control of the raw material and the processing and storage conditions are warranted.

Palm oil

In recent years, renewed attention has been directed to red palm oil as a rich source of bioavailable provitamin A. In Brazil, red palm (*Elais guineensis*) production has expanded for other reasons - e.g. as raw material for fractioning oleins and stearins, producing natural fat with diverse applications in the food industry, without the use of hydrogenation. The carotenoid extract can be an important by-product. Oil extracted from the sterilized commercial cultivar Tenera had 64 µg/g 13-*cis*-α-carotene, 94 µg/g trans-α-carotene, 129 µg/g 13-*cis*-ß-carotene, 229 µg/g *trans*-ß-carotene and 53 µg/g 9-*cis*-ß-carotene (45). This abundant carotenoid supply is unfortunately destroyed during refining of the oil. Recently, however, several countries are recovering the carotenoids during oil processing to serve as provitamin A source.

In the state of Bahia, crude red palm oil has been a common ingredient in local cuisine. As Bahian recipes become more popular in other parts of the country, so does red palm oil. Oil from other palm fruits can also be produced and serve as excellent sources of carotenoids.

With such a diversity of sources, Latin America has the daunting task of analyzing and utilizing these sources to promote the health of the population. If successful, such an effort will certainly be very rewarding.

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REFERENCES

1. Hiani PA, Penteado MVC. Carotenóides e valores de vitamina A do fruto e da farinha de bocaiúva (*Acrocomia mokayáyba* Barb. Rodr.) do Estado de Mato Grosso do Sul. Rev. Farm Bioquim Univ S Paulo 1989; 25:158-68.
2. Godoy HT, Rodriguez-Amaya DB. Buriti (*Mauritia vinifera* Mart), uma fonte riquíssima de pró-vitamina A. Arq Biol Tec 1995;38:109-20.
3. Godoy HT, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of Brazilian loquat (*Eriobotrya japonica* Lindl.). Arch Latinoamer Nutr 1995; 45:336-9.
4. Godoy HT, Rodriguez-Amaya DB. Composição de carotenóides em nectarina (*Prunus persica*) brasileira. Rev Inst Adolfo Lutz 1998;57: 73-9.
5. Rodriguez-Amaya DB, Kimura M. Carotenóides e valor de vitamina A em cajá (*Spondias lutea*). Cienc Tecnol Aliment 1989;9: 148-62.
6. Cecchi HM, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of fresh and pasteurized cashew-apple (*Anacardium occidentale L.*) juice. J Food Sci 1981;46:147-9.
7. Padula M, Rodriguez-Amaya DB. Characterisation of the carotenoids and assessment of the vitamin A value of Brazilian guavas (*Psidium guajava L.*). Food Chem 1986;20:11-9.
8. Rodriguez-Amaya DB, Godoy HT, Mercadante AZ, Ramos

DMR. Carotenoid composition of underexploited Brazilian fruits. Proceedings of the 9th World Congress of Food Science and Technology; 1995 July 30-Aug 4; Budapest: International Union of Food Science and Technology, 1995.

9. Rodriguez-Amaya DB, Bobbio PA, Bobbio FO. Carotenoid composition and vitamin A value of the Brazilian fruit *Cyphomandra betacea*. Food Chem 1983;12:61-5.
10. Mercadante AZ, Rodriguez-Amaya DB, Britton G. HPLC and mass spectrometric analysis of carotenoids from mango. J Agric Food Chem 1997;45:120-3.
11. Mercadante AZ, Rodriguez-Amaya DB. Effects of ripening, cultivar differences, and processing on the carotenoid composition of mango. J Agric Food Chem 1998;46:128-30.
12. Agostini TS, Cecchi HM, Godoy HT. Composição de carotenóides no marolo *in natura* e em produtos de preparo caseiro. 13. Ciênc Tecnol Aliment 1996;16:67-71.
13. Kimura M, Rodriguez-Amaya DB, Yokoyama SM. Cultivar differences and geographic effects on the carotenoid composition and vitamin A value of papaya. Lebensm Wiss u Technol 1991;24: 415-8
14. Godoy HT, Rodriguez-Amaya DB, Tavares CA. Composição de carotenóides de pêssego e nectarina produzidos na América do Sul. Proceedings of the I Congreso de la Federación Farmacéutica Sudamericana; 1993 Nov 4-7; Montevideo: Asociación de Química y Farmacia del Uruguay, 1993.
15. Cavalcante ML, Rodriguez-Amaya DB. Carotenoid composition of the tropical fruits *Eugenia uniflora* and *Malpighia glabra*. In Charalambous G, editor. Food science and human nutrition. Amsterdam: Elsevier Science Publishers, 1992: 643-50.
16. Yuyama LKO, Favaro RMD, Yuyama K, Vannucchi H. Bioavailability of vitamin A from peach (*Bactris gasipaes* H.B.K.) and from mango (*Mangifera indica* L.) in rats. Nutr Res 1991;11:1167-75.
17. Mariath JGR, Lima MCC, Santos LMP. Vitamin A activity of buriti (*Mauritia vinifera Mart*) and its effectiveness in the treatment and prevention of xerophthalmia. Am J Clin Nutr 1989;49:849-53.
18. Gross J. Pigments in fruits. London: Academic Press, 1987.
19. Rodriguez-Amaya DB. Nature and distribution of carotenoids in foods. In Charalambous G, editor. Shelf-life studies of foods and beverages. Chemical, biological, physical and nutritional aspects. Amsterdam: Elsevier Science Publishers,1993:547-89.
20. Bonifácio MTES, Morais SB, Sylos CM. Carotenóides em três variedades brasileiras de frutas cítricas. Programa e Resumos. Congresso Latino-Americano de Carotenóides em Alimentos; 1998 Sept 14-17; Campinas: PRONEX-Carotenóides.
21. Mercadante AZ, Britton G, Rodriguez-Amaya DB. Carotenoids from yellow passion fruit (*Passiflora edulis*). J Agric Food Chem 1998;46: 4102-6.
22. Murillo EF, Arce VC, Batista B. El plátono (musa AAB), principal fuente de carotenoides en la dieta Panameña. Programa e Resumos. Congresso Latino-Americano de Carotenóides em Alimentos; 1998 Sept 14-17; Campinas: PRONEX-Carotenóides.
23. Siefermann-Harms D, Hertzberg S, Borch G, Liaaen-Jensen S. Lactucaxanthin, an ε,ε-carotene-3,3'-diol from *Lactuca sativa*. Phytochem 1981;20: 85-8.
24. Ramos DMR, Rodriguez-Amaya DB. Determination of the vitamin A value of common Brazilian leafy vegetables. J Micronutr Anal 1987;3: 147-55.
25. Kimura M, Rodriguez-Amaya DB, Godoy HT. Assessment of the saponification step in the quantitative determination of carotenoids and provitamins A. Food Chem 1990; 35:187-95.
26. Minazzi-Rodrigues RS, Penteado MVC. Carotenóides com atividade pró-vitamínica A em hortaliças folhosas. Rev Farm Bioquím Univ S Paulo 1989; 25:39-52.
27. Godoy HT, Rodriguez-Amaya DB. Occurrence of cis isomers of provitamins A in Brazilian vegetables. J Agric Food Chem 1998;46:3081-6
28. Almeida-Muradian LB, Fiorini F, Penteado MVC. Provitamin A evaluation of external and internal leaves of cabbage (*Brassica oleracea* L. var. *capitata* L.). Cienc Tecnol Aliment 1995;15:108-11.
29. Penteado MVC, Minazzi-Rodrigues RS, Almeida LB. Carotenóides e atividade pró-vitamínica A de folhas de hortaliças consumidas no Norte do Brasil. Rev Farm Bioquim Univ S Paulo 1986;22:97-102.
30. Mercadante AZ, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of some native Brazilian green leafy vegetables. Int J Food Sci Technol 1990;25:213-9.
31. Mercadante AZ, Rodriguez-Amaya DB. Carotenoid composition of a leafy vegetable in relation to some agricultural variables. J Agric Food Chem 1991;39:1094-7.
32. Almeida-Muradian LB, Popp V, Farias MP. Provitamin A activity of Brazilian carrots: Leaves and roots, raw and cooked and their chemical composition. Ciênc Tecnol Aliment 1997;17:120-4.
33. Molina-Cruz A, Wohlers LMC, Bressani R. Redescubrindo el valor nutritivo de las hojas de chaya (*Cnidoscolus aconitifolius; Euphorbiaceae*). Ciencia en Accion 1997;3:1-4.
34. Arima HK, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of commercial Brazilian squashes and pumpkins. J Micronutr Anal 1988;4:177-91.
35. Arima HK, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of a squash and a pumpkin from Northeastern Brasil. Arch Latinoamer Nutr 1990;40:284-92.
36. Bianchini R, Penteado MVC. Carotenóides de pimentões amarelos (*Capsicum annuum* L.). Caracterização e verificação de mudanças com o cozimento. Ciênc Tecnol Aliment 1998;18:283-8.
37. Tavares CA, Rodriguez-Amaya DB. Carotenoid composition of Brazilian tomatoes and tomato products. Lebensm Wiss u Technol 1994; 27:219-24.
38. Mejia LA, Hudson E, Gonzalez de Mejia E, Vasquez F. Carotenoid content and vitamin A activity of some common cultivars of Mexican peppers (*Capsicum annuum*) as determined by HPLC. J Food Sci 1988; 53: 1448-51.
39. Almeida LB, Penteado MVC. Carotenóides com atividade pró-vitamínica A de cenouras (*Daucus carota* L.) comercializadas em São Paulo, Brasil. Rev Farm Bioquim Univ S Paulo 1987; 23:133-41.
40. Penteado MVC, Almeida LB. Ocorrência de carotenóides em raízes de cinco cultivares de mandioca (*Manihot esculenta* Crantz) do Estado de São Paulo. Rev Farm Bioquim Univ S Paulo 1988; 24:39-49.

41. Almeida LB, Penteado MVC. Carotenóides e valor pró-vitamínico A da mandioquinha (*Arracacia xanthorrhiza* Bancr.) consumidas em São Paulo. Rev Farm Bioquim Univ S Paulo 1987; 23:52-7.
42. Almeida-Muradian LB, Penteado MVC. Carotenoid and provitamin A value of some Brazilian sweet potato cultivars (*Ipomoea batatas* Lam.). Rev Farm Bioquim Univ S Paulo 1992; 28: 145-54.
43. Cecchi HM, Rodriguez-Amaya DB. Carotenóides e valor de vitamina A em suco de maracujá processado. Cienc Cult 1981;33:72-6.
44. Tavares CA, Rodriguez-Amaya DB (*unpublished results*).
45. Trujillo-Quijano JA, Rodriguez-Amaya DB, Esteves W, Plonis GF. Carotenoid composition and vitamin A value of oils from four Brazilian palm fruits. Fat Sci Technol 1990; 92: 222-6.

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Necesidades de investigación en carotenoides en América Latina

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RESUMEN. Muchos de los fitoquímicos bioactivos son muy importantes para mantener un buen estado de salud y de ellos los carotenoides han sido los más estudiados. Se conocen alrededor de 637 y se ha propuesto que posiblemente 70 de ellos puedan tener un papel en la salud humana. En cortes de cerebro humano se han encontrado 16 en relativamente alta cantidad. La mayoría de los estudios encuentran relación entre los carotenoides y varias enfermedades de tipo crónico no transmisibles, sobre todo con ciertos tipos de cáncer, enfermedades aterosclerósicas y enfermedades degenerativas del ojo. Esta relación esta mediada por los genes y la edad. Los carotenoides tienen interés científico y también económico para los países Latinoamericanos, por lo que se propone hacer análisis en los productos tropicales, frutas y verduras más comunes, lo mismo que hacer estudios sobre su biodisponibilidad. Se sugiere un trabajo coordinado en el que se determinen los 7 carotenoides más relacionados con la salud en 40 ó 50 productos tropicales, lo mismo que comenzar a evaluar su disponibilidad metabólica. Se considera asimismo posible hacer estudios epidemiológicos basados en las poblaciones consumidoras de algunos alimentos ricos en carotenos para hacer análisis de riesgos comparativos. En algunos países de Latinoamérica, con el patrocinio de FAO e Infoods, ya se han llevado a cabo algunas reuniones y cursos para iniciar este importante trabajo, lo mismo que para completar las tablas de composición de alimentos y hacer comparaciones entre países.

Palabras clave: Carotenoides, investigación, funciones, antioxidantes, enfermedades crónicas.

SUMMARY. Investigation needs on carotenoids in Latin America. Many recent papers show the important role of bioactive phytochemicals to mantain a good health status. Among them the carotenoids are the best known. About 637 have been described and possibly 70 of them could have an important role in human health, 16 have been found in human brain in high amounts. Most of the studies have found relations between the carotenoids and chronic non-communicable diseases like several types of cancer, atherogenic disease and some degenerative pathology of the eye. This relation is mediated by genes and age. Studies of carotenoids are of scientific and economic interest for Latin America as many tropical products are high sources of these compounds. Therefore the first task is to analyze them and iniciate some evaluation on its metabolic availability. A coordinated regional work is proposed, in which 40 or 50 fruits and vegetables are analyzed in terms of the seven carotenoids most related to human health. At the same time it will be important to start epidemiological studies that will compare groups with different levels of consumption of fruits and vegetables and make chronic disease risk analysis. In some countries of the Latin American region, with the support of FAO and INFOODS, some courses and meetings are taking place so that in a short time period the carotenoid composition of the important regional foods will be completed and a carotenoid regional food composition table be published.

Key words: Carotenoids, research, functions, antioxidants, chronic diseases.

Hasta muy recientemente, quizá en los últimos 15 años, se ha reconocido la importancia de la gran familia de los llamados fitoquímicos bioactivos. Son conocidos en el laboratorio desde la primera mitad del siglo, cuando hubo una especie de fiebre por descubrir nuevas vitaminas y varios de los ahora fitoquímicos fueron propuestos como tales porque tenían funciones en especies de laboratorio, pero por no ser esenciales en el humano fueron descartados y dejados en el olvido por muchos años.

En base a los resultados que muchos investigadores están publicando recientemente en casi todas las revistas de nutrición, se puede proponer lo que los autores en México denominan como "nueva nutrición" o sea una rama de la ciencia en expansión, que no solo considera a los nutrientes y sus necesidades, los alimentos que los contienen y la forma de integrar dietas, sino la ciencia que evite la desnutrición y también las enfermedades crónicas como la aterosclerosis, la obesidad, la hipertensión, el cáncer y muchas más, por lo tanto que considera a los antioxidantes y otros fitoquímicos en todo el proceso nutricional, desde su conocimiento intrínseco, su contenido en los alimentos, las cantidades aconsejadas y sobre todo como integrar una alimentación agradable, incluyendo, en los casos en los que se necesitan a varios suplementos.

Esta nueva nutrición se debe basar en mucho trabajo científico porque aunque el público requiere de una información fácil y solo en alimentos, quizá en platillos preparados, el especialista debe proponerlos con el conocimiento de los 70 ó más fitoquímicos con actividad biológica conocidos actualmente: sus funciones, las cantidades necesarias, los alimentos que por su contenido se pueden llamar "funcionales", sus interacciones y por supuesto la relación que pueden tener en cada persona o familia en función de sus riesgos de padecer

enfermedades crónicas. Lo que a largo plazo se propone es llegar a poder definir bien las interacciones entre los genes de cada grupo humano y los compuestos químicos de su comida diaria para, al compatibilizarlos, propiciar una buena salud por mucho tiempo. Por más lejano que esto se vea, la verdad es que ya está muy cerca, sobre todo por la intensa investigación en los llamados "nutracéuticos" y el gran mercado que de ellos ya existe, por varios miles de millones de dólares. Todo este nuevo campo llega al grado que, ya en la situación actual, los nutricionistas deben participar en forma muy destacada en la medicina preventiva y cuando se sepa un poco más, es muy probable que deban ser los que la realicen en su mayor parte.

Dentro del complejo panorama de los fitoquímicos bioactivos se destacan por su importancia los carotenoides, una familia química fundamental de las plantas, ya que son parte del cloroplasto, con la función de absorber fotones y evitar que las hojas se quemen por efecto de las radiaciones solares. Antes se pensaba que eran parte solo del mundo vegetal, sin ningún papel en los animales y menos en el hombre. La situación real es diferente, los carotenos y carotenoides posiblemente son más importantes para el hombre y demás primates, que para los demás animales, porque para ellos están resultando fundamentales para la preservación de la salud a lo largo de la vida y sobre todo para las épocas tardías.

En el humano y en los demás animales arbóreos varios carotenoides tienen también la función de captar fotones ya que ayudan a prevenir la degeneración macular de la retina y posiblemente también las cataratas y otros padecimientos degenerativos del ojo, aunque su función más conocida también se relaciona con la luz y es su potencial de formar el retinol, la vitamina A y por lo tanto la rodopsina, la sustancia activa de la reacción química en los conos y bastones que generan el estímulo visual en el sistema nervioso central y que permite la visión. En este sentido, a varios carotenos se les clasifica como provitaminas por ser formadores de la vitamina A.

Lo importante para la nueva nutrición, es que los carotenos y los carotenoides no solo captan fotones, sino también electrones y por lo tanto son antioxidantes muy particulares. En este aspecto es en el que pueden ser fundamentales para el hombre y los primates, más que para otros animales. Es muy claro que cada vez que se profundiza más en el estudio de los fitoquímicos bioactivos más se llega a la idea de que todavía la fisiología humana corresponde a la de un primate arbóreo, comedor de hojas y frutas.

No solo anatómicamente el hombre tiene un estómago enorme para moler hojas, un gran hígado y un intestino delgado de 7 u 8 metros para absorber nutrientes difíciles de obtener y un intestino grueso que es una cámara de fermentación con capacidad de muchos kilos, sino fisiológicamente las células humanas son moduladas y cada una de ellas es afectada por multitud de compuestos propios de las plantas. Este es seguramente un papel fundamental de los carotenoides, que no son en si mismos esenciales, solo cuando no se come vitamina A preformada, sino son compuestos funcionales, moduladores del metabolismo intermedio, de la comunicación intercelular y del funcionamiento químico de los ribosomas y otras partes de la célula. Hasta cierto punto pueden ser sustituidos, pero si no se consumen, con el tiempo se hace aparente su falta. Seguramente es por esto que el consumo deficiente de carotenoides, familia en la que se puede incluir también a los retinoides, tiene tanta relación con la presencia de enfermedades crónicas.

Los carotenoides están en todas las plantas, en sus partes verdes y sobre todo en las amarillas y colores semejantes, por ejemplo naranja y algunas rojas, como el caso del jitomate. El color depende de las dobles ligaduras existentes. Las frutas y las flores tienen mayor diversidad de carotenoides porque los han desarrollado también para tener colores atractivos. Cada año se descubren nuevos compuestos y en 1998 había 637 identificados. Solamente en las diferentes variedades de chiles (capsicum) se encuentran 58. Por el momento se considera que quizá 70 carotenoides y retinoides pueden tener un efecto fisiológico en los humanos, aunque 7 han sido los más estudiados: los α-, ß- y γ–caroteno, el licopeno, la zeaxantina, la luteina, la astaxantina y la ß-criptoxantina, y en los que se ha demostrado una alta capacidad antioxidante, porque secuestran oxígeno singulete y bloquean varias reacciones de los radicales libres. También absorben fotones y en esta función la luteina y la zeaxantina han demostrado no solo prevenir sino también mejorar las lesiones maculares retinianas que se presentan en las personas de edad (degeneración macular). Algunos como el ß- y el α-caroteno tienen actividad vitamínica (mencionada desde 1:4 hasta 1:26 por diversos investigadores), otros fortalecen el sistema inmunológico y la mayoría, quizá los 70 carotenoides mencionados, a través de fortalecer la comunicación intercelular y el metabolismo de las membranas, inhiben el desarrollo de varios cánceres y la presencia de tromboembolias cardiovasculares y otros padecimientos crónicos. Varios retinoides también han sido involucrados en esta importante función.

En estudios doble-ciego, con control de placebo, las personas con angina de pecho pueden prevenir hasta en un 50% las trombosis graves con el uso de ß-caroteno (1). Los mecanismos que se invocan para explicar su acción es su capacidad de capturar el oxígeno-singulete o la estimulación de la función inmune, combinados con la acción local del efecto de ayudar al establecimiento de la comunicación intercelular cuando esta está bloqueada por acción de las grasas inadecuadamente transportadas (2), tal es el caso de varios de los carotenoides que actúan como potentes antioxidantes hidrofóbicos en el plasma y en los tejidos (4) o sea que previenen la formación de hidroxiperóxidos en los fosfolípidos de los LDL y por lo tanto reducen el riesgo de daño causado por este tipo de radicales libres.

Se sabe que el nivel de consumo de frutas y verduras se asocia directamente con disminuir el riesgo de cáncer en general y específicamente de los de pulmón, mama y próstata, pero en contraste es frecuente que en estudios de suplementación con ß-caroteno no se encuentre efecto alguno sobre el riesgo de cáncer de pulmón y a veces también con el de mama. Esta situación sugiere que pueden ser una acción combinada de varios carotenoides por lo que para la prevención del cáncer es más aconsejable por el momento el consumo de verduras y frutas y no de suplementos individuales.

De todas las áreas de estudio la que promete más, aunque es difícil, es la interacción entre algunos carotenos con el cáncer. No se sabe claramente qué carotenos y en cuáles condiciones pueden ser más útiles. Por ejemplo se sabe que el ß-caroteno puede aún curar lesiones precancerosas de boca causadas por masticar tabaco, pero también se ha encontrado que no es positivo para prevenir el cáncer de pulmón en los fumadores (3). Esto se puede deber a la interacción entre diversos carotenos y no siempre el ß-carateno puede ser el indicado.

Quizá un problema básico que explicaría la inconsistencia de algunos estudios está en la absorción y transporte de los diferentes carotenoides. El ß-caroteno es el más conocido y el que está presente en la mayoría de las verduras y frutas, por lo tanto es al que además de considerársele una provitamina A es al que se le atribuyen más propiedades antioxidantes. Es por ello que es el más reportado como agente en la prevención de los cánceres mencionados y de las enfermedades cardiovasculares. Existen varios estudios que muestran la importante interacción entre el ß-caroteno y varios de los demás carotenoides, por ejemplo Nieremberg et al. (5) después de suplementar ß-carotenos por dos años a un grupo de personas encontraron grandes incrementos en la concentración sérica de lycopeno y α-caroteno. También hubo aumentos en la luteína/zeaxantina y en la ß-criptoxantina, pero no significativos. Esto quiere decir que el ß-caroteno ayuda a la absorción o el metabolismo del licopeno y del α-caroteno pero no al de las xantofilas (o sea los que contienen oxígeno).

En otros estudios también se encuentra una muy interesante interrelación entre todos los carotenoides, así Van Den Berg y Van Vliet (6) encuentran que la luteina afecta negativamente a la absorción del ß-caroteno cuando se consumen simultáneamente. No afecta a los otros carotenoides ni a su partición para producir vitamina A. También se ha encontrado que el ß-caroteno inhibe la absorción de la cantaxantina en humanos.

Otro hecho importante es que las concentraciones séricas después de la suplementación con varios carotenoides, variaron inversamente con el índice de masa corporal, hábito de fumar, consumo de productos animales y edad. La absorción de ß-caroteno también en función de la concentración sérica del mismo fitoquímico (6).

Se han encontrado relaciones epidemiológicas entre el consumo de carotenos, especialmente de ß-caroteno, con enfermedades tromboembólicas cardio-vasculares, con cáncer y parcialmente con algunos problemas crónicos de ojos, sobre todo con la prevención de cataratas y de la degeneración macular de retina, pero hay que recordar que existen 55 síndromes crónicos que tienen alguna relación con dieta, quizá por la función antioxidante de algunos elementos, por lo que se requiere más investigación sobre la posible participación de los carotenos. Por esto los doctores Britton y Pfander han insistido en la necesidad de trabajar en el área de estructura y biodisponibilidad (7).

Es posible que varios carotenoides sean una pieza importante del problema de salud planteado por la triada: genes, dieta y edad. Se sabe que una mala alimentación va desgastando la acción de los genes y que tarde o temprano, en los genéticamente susceptibles, aparece una alteración anatómica o funcional que progresivamente inhabilita a las personas y propicia las enfermedades crónicas graves. De los factores dietéticos los más constantes se relacionan al efecto positivo de frutas y verduras y de estas las más eficientes son las de colores verde o amarillo fuertes, lo que puede significar que los carotenoides pueden ser los principales actores (8).

Un tema de mucho interés es el relativo al hecho que de los 55 síndromes crónicos que se presentan como consecuencia del triángulo genes-dieta-edad, por lo menos 8 son cerebrales y van desde las graves enfermedades de Alzhaimer y Parkinson, hasta los simples temblores o la perdida parcial de memoria debidos a la edad. De varios de ellos ya se sabe que el exceso de oxidaciones bloquea la producción de algunos neurotransmisores. La pregunta seria: Qué papel tienen en estos importantes síndromes los diferentes carotenoides? Se sabe que para la protección de la retina contra la degeneración macular, un típico tejido nervioso, los carotenoides zeaxantina/luteína son muy activos, ya que no solo ayudan a la prevención sino también al tratamiento, lo que sugiere que algo semejante puede ser posible para otros tejidos o grupos celulares del sistema nervioso.

En análisis muy precisos de cerebros humanos (9) se encontraron que en todos había cantidades importantes de 16 carotenoides. Ocho bien identificados (luteína, zeaxantina, anhidroluteína, α-criptoxantina, ß-criptoxantina, α-caroteno, ß-caroteno *cis-* y *trans-* y licopeno *cis-* y *trans-* y 8 xantofilas que por el momento han sido difíciles de identificar. Este contenido tan variado puede suponer que pueden tener algunas funciones importantes.

Se puede considerar que toda el área de fitoquímicos bioactívos y en especial la de los carotenos es relativamente nueva en investigación, aunque ya se están publicando varios cientos de trabajos por año, pero existen varios aspectos en los que Latinoamérica puede hacer grandes aportaciones (10). La riqueza en carotenoides es mucho mayor en las plantas tropicales ya que se sabe que el 95% de la variabilidad biológica del mundo está en los trópicos, por lo que seguramente para los primates como para el hombre los carotenos tropicales y sus mezclas pueden ser más favorables para la salud.

Un tema prioritario para Latinoamérica es el análisis de contenido en los principales carotenoides de los principales productos tropicales, como pueden ser el mango, la papaya y la piña incluyendo sus distintas variedades y en diverso grado de maduración, provenientes de las distintas regiones, porque se sabe que estas condiciones influyen, pero simultáneamente se deben agregar dos áreas más, la de disponibilidad metabólica de los diferentes carotenoides o sea su absorción y retención, pero sobre todo extender los estudios a especies de frutas y verduras mal conocidas en los países y ciudades de alta capacidad económica, pero con potencial nutricional y económico.

La cantidad de trabajo necesaria para determinar los principales carotenoides de quizá 40 ó 50 frutas y verduras tropicales, en sus diferentes condiciones y sobre todo para conocer algunos aspectos sobre su biodisponibilidad, es ya muy grande como para en el momento pensar en otras áreas de interés, pero existen y sobre todo con grandes perspectivas para la región, por ejemplo en el área clínica y epidemiológica. Existen grupos humanos en el trópico que consumen grandes cantidades de algunas especies ricas en carotenos y ellos pueden constituir grupos naturales de comparación sobre los efectos fisiológicos y para hacer un análisis de riesgos comparativo. También existen diferencias regionales y entre grupos rurales y urbanos, lo mismo que se pueden planear estudios de intervención y cohortes, todos ellos dirigidos aprobar el papel funcional de varios de los alimentos tropicales.

Falta mucha información al respecto de las verduras de hoja verde que no son muy conocidas fuera de sus regiones originarias, lo mismo se podría decir de las distintas variedades de los alimentos americanos que si se han divulgado fuera, como muchas variedades de papas, camotes, tomates, raíces amarillas, chiles, etc. Estos estudios se deberían combinar con la investigación agrícola sobre selección de variedades, hibridización, etc. que podrían dar lugar a semillas de variedades de alto contenido y alta disponibilidad de carotenos, seleccionados como verdaderas variedades "funcionales" por su valor nutricional, económico y social. La tecnología de alimentos podría participar con estudios sobre industrialización de los productos y su comercialización a gran escala. A este respecto se está comenzando a saber que algunos procesos pueden facilitar la biodisponibilidad de los carotenoides.

Hace falta investigación en tecnología de alimentos sobre la forma de proteger los carotenos y otros fitoquímicos bioactivos durante los procesos de almacenamiento, transporte e industrialización. De hecho no se sabe mucho sobre sus cambios en relación a los distintos productos y variedades durante su proceso de maduración y simplemente durante su almacenamiento. Hacen falta estudios colaborativos para el análisis de frutas, verduras y algas, lo mismo que también en el caso de insectos, mariscos y crustáceos que no sintetizan los carotenos pero los acumulan y a veces los modifican químicamente.

Debido a que la investigación en carotenoides tiende a ser difícil y cara es indispensable la cooperación entre universidades, institutos y laboratorios de los distintos países. Ya LATINFOODS ha dado los primeros pasos. En México y en el Caribe han habido dos reuniones y dos cursos especializados, con el interés de estandarizar metodologías y distribuir el trabajo. La subregión de México y Caribe es origen histórico de muchas raíces, frutas, verduras y otros alimentos de interés mundial. Se espera que el esfuerzo desplegado por las instituciones, universidades e industrias de la región, alcancen pronto logros importantes en el estudio de los alimentos ricos en la gran diversidad de carotenoides existentes, pero desconocidos para el resto del mundo, lo mismo que se mejoren las tablas de composición de alimentos con la inclusión de los diversos carotenoides.

REFERENCIAS

1. Kohlmeier L, Hasting SB. Epidemiological evidence of a role of carotenoids in cardiovascular disease prevention. Am J Clin Nutr 1995; 62 (suppl):137ÖS.
2. Santos MS. et al. ß-carotene-induced enhancement of natural killer cell activity in elderly men: and investigation of the role of cytokines. J Clin Nutr 1998; 68: 164.
3. Garewal HS. Emerging role af ß-carotene and other antioxidant nutrients in prevention of oral cancer. Arch Otolaringol Head Neck Surg 1995; 121: 141.
4. Sun S. et al. Anti-tumor activity of astaxanthin on meth-A tumor cells and its mode of action. Summary 5595, FASEB J, Abstracts of experimental biology 98, San Francisco CA.
5. Nieremberg DW, Stukel TA, Mott LA, Greenberg ER. Steady state serum concentration of alpha-tocopherol not altered by supplementation with oral ß-carotene. J Natl Cancer Inst 1994; 86: 117.
6. van den Berg H, van Vliet T. Effects of simultaneous simple oral doses of ß-carotene with lutein or lycopene on the ß-carotene and retinyl ester responses in the triacylglycerol-rich lipoprotein fraction in men. J Clin Nutr 1998; 68: 82.
7. Muñoz de Chávez M, Rocabado F, López Franchini J, Chávez A. La alimentación y las enfermedades crónicas. México: INN, SSA y PHAO-NU, 1992.
8. Potter J, Chávez A. et al. Food, nutrition and the prevention of cancer. Washington DC: WCRI/AICR, 1997.
9. Craft N, Garnett K, Herdley-White ET. et al. Carotenoids. tocopherols and vitamin A in human brain. Summary 5601 FASEB J., San Francisco, April 1998.
10. Muñoz de Chávez M, Chávez A. Nutrición y cáncer en Latinoamérica, 1998.

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LATINFOODS y su rol en la generación y compilación de datos para América Latina

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RESUMEN. LATINFOODS es la organización Latinoamericana filial de INFOODS involucrada en la generación de datos en composición de alimentos, su compilación, los métodos de análisis y los usuarios de estos datos. La UNU y FAO son las organizaciones que han apoyado las actividades de esta red a través de la realización de talleres de discusión y capacitación, cursos específicos, simposios de análisis de la gestión y planes de acción a futuro. Una de las acciones concretas ha sido la edición preliminar de la Tabla de Composición de Alimentos de América Latina (1998). Entre las prioridades de LATINFOODS está la generación de datos en diversos nutrimentos, entre los cuales los pigmentos carotenoides ocupan un lugar fundamental. La razón original de su estudio e incorporación obligada en las Tablas de Composición de Alimentos de todos los países, estaba en estrecha relación con su actividad como provitamina A o equivalentes de retinol como era la expresión clásica en dichas tablas. En este contexto, el ß-caroteno por su mayor actividad biológica era el que se determinaba de preferencia. Se sabe que en la naturaleza existe tanto en el reino vegetal como animal un sinnúmero de pigmentos carotenoides que cumplen diversos roles, además de su clásico poder de dar a los productos alimenticios colores atractivos que van desde el amarillo pasando por el naranja hasta el rojo. El interés en conocer la composición de los diferentes carotenos y xantofilas de los alimentos naturales y procesados se ha acrecentado en los últimos años por su actividad biológica como posible antioxidante *in vivo* en humanos. Esta circunstancia ha dado un nuevo impulso al estudio de los pigmentos carotenoides presentes en los alimentos más allá del ß-caroteno. Los métodos de determinación de carotenoides es otro tema prioritario para LATINFOODS. Se ha hecho un curso patrocinado por FAO, pero se requiere la realización de nuevas actividades concretas, dada la complejidad del tema que requiere de vasta experiencia y que lidera en Brasil la Dra. Delia Rodríguez-Amaya. Esperamos que la próxima edición de la Tabla de Composición de Alimentos para América Latina que se publicará en dos años más, contenga el máximo de información sobre estos componentes. Nuestra Región es privilegiada en cuanto a alimentos nativos que evidentemente son potencialmente excelentes fuentes de carotenoides. Considero un deber para todos nosotros, el abocarnos a su estudio a la brevedad por la importancia que ello revista a todo nivel.

Palabras clave: LATINFOODS, análisis, Tabla de Composición de Alimentos.

SUMMARY. LATINFOODS and its role in the generation and compilation of data for Latin America. LATINFOODS is the Latin American organization, affiliated to INFOODS, involved in the generation and compilation of data on the composition of foods, and with the methods of analysis and the users of the data. UN and FAO are the organizations which have supported the activities of this network, through the realization of workshops, specific courses, symposia analyzing present situation and future plans of action. One of the concrete actions has been the preliminary edition of the Food Composition Table of Latin America (1998). Among the priorities of LATINFOODS is the generation of data on various nutrients, among which the carotenoid pigments occupy a fundamental place. The original reason for their determination and their compulsory inclusion in food composition tables of all countries was related directly to their provitamin A activity or retinol equivalents as classically expressed in the mentioned tables. In this context, ß-carotene, because of its higher biological activity, was preferentially determined. It is known that in nature, both in the plant and in the animal kingdom, there are numerous carotenoid pigments that play diverse roles, other than the classic ability to confer attractive color to foodstuffs, ranging from yellow, passing through orange to red. The desire to know the composition of the different carotenes and xanthophylls in fresh and processed foods has increased in recent years because of their biological activity as antioxidants *in vivo* in humans. This circumstance has given new impulse to the study of carotenoids in foods other than ß-carotene. Methods to determine carotenoids constitute another priority for LATINFOODS. A course sponsored by FAO has been carried out, but the realization of new concrete activities is necessary, given the complexity of the subject which requires vast experience and is led in Brazil by Dr. Delia Rodriguez-Amaya. It is hoped that the next edition of the Food Composition Table for Latin America, which will be published in two years, will have the maximum information about these compounds. Our region is privileged with native foods which are potentially excellent sources of carotenoids. It is our duty to carry out this investigation as soon as possible, considering its importance at all levels.

Key words: LATINFOODS, analysis, Food Composition Table.

Reflexiones ...
El sol es amarillo y es nuestra fuente de vida.
El oro es amarillo y sigue siendo el metal más apreciado.
Nuestros alimentos amarillos están a la mano, pero nos empeñamos en ignorarlos.
Es hora de redescubrirlos, su color amarillo es símbolo de vida, salud y bienestar para todos.

LATINFOODS es la organización latinoamericana filial de INFOODS que agrupa a los investigadores de la Región involucrados en la generación de datos en composición de alimentos, su compilación, los métodos de análisis y los usuarios de estos datos.

La UNU y FAO son las organizaciones que han apoyado las actividades de esta red a través de la realización de talleres de discusión y capacitación, cursos específicos, simposios de evaluación de la gestión y desarrollo de planes de acción a futuro.

Una de las acciones concretas ha sido la edición preliminar de la Tabla de Composición de Alimentos Latinoamericana entregada en el simposio de LATINFOODS, congreso SLAN Guatemala 1998. Esta edición se generó en el Subcentro SAFOODS con sede en Chile, Universidad de Chile, como resultado del trabajo integrado de los coordinadores de LATINFOODS de cada país en carotenoides aplicados a alimentos grasos y no grasos. Se ha hecho un curso patrocinado por FAO, pero se requiere la realización de nuevas actividades concretas, dada la complejidad del tema que requiere de vasta experiencia y que lidera en Brasil la Dra. Delia Rodríguez-Amaya de la Universidad Estadual de Campinas. Esta proposición está incluida en el Proyecto Regional sobre Composición de Alimentos que se está elaborando por el presidente y vice-presidente de LATINFOODS para ser sometido a consideración de FAO.

Se ha estimulado el trabajo integrado entre grupos de investigadores de Brasil, Argentina y Chile sobre el tema: "Fuentes de pigmentos naturales con actividad biológica", presentado como proyecto de investigación a organismos internacionales. Nuestra región es privilegiada en cuanto a alimentos nativos que potencialmente son excelentes fuentes de carotenoides.

Como presidente de LATINFOODS espero que a través de este primer Congreso dedicado al tema, los investigadores de los países participantes establezcan programas de investigación específicos para generar información actualizada sobre el aporte de carotenos y xantofilas de sus alimentos, tanto naturales como procesados. Considero un deber para todos nosotros, el abocarnos a su estudio a la brevedad, por la importancia que ello reviste a todo nivel.

Se propone tomar el acuerdo de iniciar entre los investigadores de la región un estudio colaborativo en relación a metodología de determinación de pigmentos carotenoides en un alimento tipo, común para todos nosotros. Este estudio debe ser dirigido por la Dra. Delia Rodríguez-Amaya con la colaboración de FAO y LATINFOODS.

Los integrantes de LATINFOODS esperamos que la próxima edición de la Tabla de Composición de Alimentos para Latinoamérica que se publicará en dos años más, contenga el máximo de información sobre estos nutrimentos, por el importante rol biológico que cumplen en nuestro organismo.

Vitamina A en Latinoamérica

Contradicción:

- Deficiencia subclínica de vitamina A en países de la región latinoamericana que normalmente tienen alimentos, especialmente frutos, que son ricos en pigmentos carotenoides que pueden proporcionar cantidades adecuadas de vitamina A.

Causas múltiples:
- Nivel educacional de la madre
- Hábitos alimentarios
- Disponibilidad de alimentos

Escasa información disponible del aporte de carotenoides de los alimentos de cada país, especialmente productos nativos.

Rol de LATINFOODS

En general, las tablas de composición de alimentos de Latinoamérica, no cuentan con información actualizada sobre aporte separado de vitamina A, carotenos y xantofilas de los diferentes alimentos naturales y procesados que son buena fuente de estos nutrientes. Los datos son antiguos y los valores pueden estar subestimados. En Chile la información sobre vitamina A y ß-caroteno proviene de estudios realizados bastante años atrás en alimentos de origen animal y vegetal, principalmente por la profesora autora de esta ponencia. Los métodos empleados en la época fueron la saponificación en caso necesario, extracción con solvente orgánico, fraccionamiento en columna de alúmina activada, y lectura posterior al espectrofotómetro a la longitud de onda correspondiente a vitamina A y ß-caroteno en caso que ambos estuvieran presente. Posteriormente, la generación de datos en ß-caroteno se interrumpió hasta que se retomó hace dos años a raíz del apoyo recibido de la Dra. Delia Rodríquez-Amaya. La actividad se ha centrado en el estudio de los pigmentos carotenoides en cascarilla de rosa mosqueta (*Rosa aff. rubiginosa*). Posteriormente, se seguirá trabajando con otros alimentos de origen vegetal y marino considerados potencialmente buenas fuentes biológicas de pigmentos carotenoides. En esta forma, se espera reiniciar la contribución a la Tabla de Composición de Alimentos Chilenos, con nueva información generada de acuerdo a los métodos aplicados actualmente. En Chile no se han efectuado estudios recientes sobre deficiencia subclínica de vitamina A, en general, la dieta chilena incluye habitualmente el consumo de frutas y verduras que aportan

pigmentos carotenoides.

Brasil es el país que más información reciente ha aportado acerca del contenido de pigmentos carotenoides de diversas frutas y verduras gracias a los aportes de todos conocidos de la Dra. Delia Rodríguez-Amaya sobre el tema.

Dentro de las políticas de investigación de LATINFOODS está el promover las siguientes acciones tendientes a mejorar la información sobre el tema.

Se ha incluido separadamente vitamina A y ß-caroteno (equivalentes de retinol), como nutrientes fundamentales en la base de datos correspondiente a la edición de la Tabla de Composición de Alimentos para Latinoamérica, lo cual es también válido para las tablas nacionales.

Se ha incentivado la generación de datos sobre pigmentos carotenoides en los países de la región atendiendo, además de su actividad como provitamina A, a su probable rol como antioxidante biológico *in vivo*, por lo que es necesario separar, identificar y cuantificar los diferentes pigmentos carotenoides presentes en los alimentos.

Se espera promover nuevos talleres con el apoyo de FAO donde se discuta y practique los métodos de extracción, purificación e identificación de pigmentos conformidad a las directrices del taller CTPD sobre composición de alimentos realizado en Chile, FAO, INTA en 1995.

Entre las prioridades de LATINFOODS está la generación de datos en diversos nutrimentos, entre los cuales los pigmentos carotenoides ocupan un lugar fundamental en cuanto a su aporte por la dieta.

La razón original de su estudio e incorporación obligada en las tablas de composición de alimentos de todos los países, estaba en estrecha relación con su actividad como provitamina A o equivalentes de retinol como era la expresión clásica en dichas tablas. En este contexto, el ß-caroteno por su mayor actividad biológica era el que se determinaba de preferencia.

Se sabe que en la naturaleza existe tanto en el reino vegetal como animal un gran número de pigmentos carotenoides que cumplen diversos roles, además de su clásico poder de dar a los productos alimenticios colores atractivos que van desde el amarillo pasando por el naranja hasta el rojo. El interés en conocer el aporte de los diferentes carotenos y xantofllas de los alimentos naturales y procesados, se ha acrecentado en los últimos años por su actividad biológica como posibles antioxidantes *in vivo* en humanos. Esta circunstancia ha dado un nuevo impulso al estudio de los pigmentos carotenoides presentes en los alimentos, más allá del ß-caroteno y su actividad como provitamina A.

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Panorama de la investigación sobre carotenoides en el Brasil. Perspectiva y necesidades

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RESUMEN. Durante las dos últimas décadas, el país produjo más de 80 trabajos científicos en carotenoides, la mayoría sobre composición, desarrollo de métodos analíticos y la influencia de varios factores sobre la composición. Diferencias entre variedades, condiciones de cultivo, de clima y grado de maduración, condiciones de almacenaje, procesamiento y preparación de alimentos figuran entre los factores que afectan la composición. Trabajos sobre biodisponibilidad y funciones y acciones en la salud vienen siendo también desarrollados y éstos deben ser incrementados. Hay un creciente interés en investigaciones sobre la degradación de los carotenoides con formación de aroma/sabor deseable o indeseable en los alimentos. En el futuro, los estudios en análisis y composición deberán continuar para incrementar la base de datos que servirán a proyectos agronómicos, nutricionales, de salud pública y biotecnológicos. Considérase fundamental continuar dando importancia máxima a la calidad de los datos generados, pues esto determina la confiabilidad de las conclusiones, no importa en qué sub-área se ubique el trabajo.
Palabras clave: Carotenoides, provitamina A, vegetales, frutas, verduras, acciones, funciones, ß-caroteno, licopeno.

SUMMARY. Research on carotenoids in Brazil. Perspective and needs. During the last two decades Brazil has produced more than 80 scientific papers on carotenoids, most of which dealing with food composition, development of analytical methodology and the factors that influence composition. Varietal differences, agricultural practices, climate and stage of maturity, as well as food storage, processing and preparation are the main influencing factors. Studies on bioavailability and the functions and actions in health have also been carried out and are expanding. Recently, interest has grown on the degradation of carotenoids with the production of either desirable or undesirable aroma/flavor in foods. For the future, analytical and compositional studies should continue to enlarge the basis for agronomic, nutritional, medical and biotechnological projects. It is fundamental that the quality of analytical data continues to receive top priority, for this can mean the difference between reliable and confounding results, regardless of the sub-area of application.
Key words: Carotenoids, provitamin A, vegetables, fruits, actions, functions, ß-carotene, lycopene

En la década del 80, el énfasis de los trabajos brasileños en carotenoides era sobre la copilación de datos de composición. Indudablemente que al mismo tiempo que se obtenían nuevas informaciones sobre la flora útil al hombre como alimento, se hacía indispensable validar metodologías y procedimientos analíticos, a fin de reunir datos confiables para el futuro. La supersimplificación de las técnicas analíticas o la utilización de un mismo procedimiento para productos muy diferentes eran operaciones realizadas con cautela y no significaban ventaja alguna antes de ser validadas científicamente. El know-how analítico se irradió del Departamento de Ciencias de Alimentos, Facultad de Ingeniería de Alimentos de la Universidade Estadual de Campinas, para diversas universidades e institutos de investigación en el país. Esta revisión tuvo como objetivo simplemente actualizar lo alcanzado hasta ahora con las investigaciones hechas en el Brasil y, por tanto, no pretendió ser una revisión crítica.

Toda la gama de trabajos puede dividirse en ocho temas, a saber: (a) validación y actualización de las metodologías analíticas, (b) confirmación y elucidación de estructuras, (c) estúdio de la composición de las fuentes, (d) influencia de algunos factores edafoclimáticos, (e) incidencia de los isómeros *cis*, (f) efecto del almacenaje, del procesamiento y la preparación doméstica, (g) la función provitamínica, y (h) el estudio de otras acciones y funciones biológicas de los carotenoides.

Técnicas analíticas y datos de composición

Con relación a las metodologías analíticas, la contribución brasileña ha sido considerable (1-11). Estos son trabajos que tuvieron gran importancia en la demostración del uso correcto de las técnicas analíticas. No es posible analizar carotenoides con éxito sin ejercer máxima atención, cuidado y celeridad, eso sin considerar el alto grado de conciencia química que se requiere. Después de leer algunos de esos trabajos, por ejemplo, se entiende por que el análisis de una calabaza madura es mucho más complejo que el de un tubérculo como la zanahoria y que el método de extracción empleado para una fruta no puede ser usado para extraer ß-caroteno de un espagueti

fortificado, antes de hacerse un estudio formal de validación. La filosofía y detalles sobre cómo realizar los análisis fueron didácticamente integrados y discutidos en cuatro publicaciones (12-15). Actualmente, un nuevo libro sobre el tema está siendo lanzado por la ILSI Press (16).

Como las propiedades biológicas (funciones y acciones) de los carotenoides dependen obviamente de sus estructuras químicas, la confirmación y elucidación de estructuras eran obstáculos que debían ser superados antes de alcanzar las metas de composición de algunos productos (17-23). En su medio natural, los carotenoides se encuentran generalmente en la configuración más estable, toda *trans*. Sin embargo, pequeñas cantidades de isómeros *cis* son cada día más y más reportadas en productos procesados y hasta *in natura*. En vista de que las actividades biológicas de los dos isómeros son diferentes, la incidencia de isómeros *cis* en ciertos productos fue también investigada (6,24-26).

Tanto el desarrollo de métodos analíticos como la confirmación de estructuras fue parte esencial para el trabajo descriptivo de los alimentos brasileños. Inicialmente por columna abierta y después por HPLC, los extensos estudios composicionales legaron al Brasil un banco de datos sobre composición de alimentos en carotenoides más grande que cualquier otro país pueda tener (27-59). La mayoría de estos estudios objetivaba describir la composición lo más completamente posible y no solamente la de aquellos carotenoides con valor provitamínico A. Puede decirse que los datos para las tablas de composición y valores vitamínicos que hoy se encuentran a disposición, resultaron como consecuencia lógica de los trabajos de composición.

Factores que influyen en la composición de carotenoides

Existía también gran interés en evaluar los factores que afectan la composición final de los alimentos. Por un lado, no se sabía con seguridad si la composición y el valor vitamínico A de un producto variaban, cuantitativa y cualitativamente, más con la maduración o con el procesamiento. Por otro lado, la tecnología de alimentos conocía la retención de nutrientes de varios tipos cuando sometidos al blanqueamiento, procesamiento y preparo doméstico, mientras que lo poco que se sabía con respecto a los carotenoides era basado en técnicas analíticas anticuadas e inexactas. Por esa razón fueron estudiados efectos edafoclimáticos, como tipo de cultivar (34-36, 41, 43, 48, 57, 67), región geográfica (42,48,60), forma de cultivo (60), y factores de almacenaje (52,61-65), procesos industriales (61-63,66,67) y condiciones de preparo doméstico (56, 57, 68, 69).

A través de esos estudios se tornó evidente que ocurren alteraciones tanto cualitativas como cuantitativas en los perfiles de composición y que las pérdidas de carotenoides debidas a la pasteurización, deshidratación y almacenamiento, cuando tales tratamientos son efectuados dentro de la racionalidad, no llegan a igualarse en magnitud a los aumentos que se registran cuando los alimentos pasan de inmaturos para maduros (32,34,67,70). Estos factores fueron presentados y discutidos en conjunto en un artículo de revisión (71), un capítulo de libro (72) y un libro (73).

Biodisponibilidad

Dentro de la actividad provitamínica, el tema de la biodisponibilidad ha provocado varias investigaciones que muestran, por ejemplo, el aumento del retinol plasmático en ratas después de consumir fuentes ricas en ß-caroteno (74,75). Sin embargo, sabiéndose que estos animales poseen un sistema de conversión por demás eficiente y teniendo en cuenta los más recientes conceptos de biodisponibilidad, lo lógico es que estos experimentos se realicen con modelos validados, o directamente con humanos. El objetivo es evitar pérdidas de tiempo y recursos al esperarse un día usar los resultados de comparación de fuentes diferentes o haciendo extrapolaciones para humanos. Dos estudios fueron realizados en humanos, uno utilizando el fruto buritĺ (76), la más rica fuente de ß-caroteno conocida (45), y otro que específicamente usó fortificación de aceite comestible (77). Estos últimos autores proponen que los aceites comestibles refinados sean fortificados con aceites ricos en ß-caroteno, como los de dendé (palma africana) y buritĺ, para atender a las necesidades de los grupos más carentes.

Otras funciones y acciones

En tratándose de las funciones y acciones de los carotenoides en el organismo, diferentes de la actividad provitamínica A, nótase que los estudios comenzaron en la década del 90, época en que los temas sobre la actividad vitamínica pasaron a ocupar un segundo plano en la investigación mundial. Moreno et al. (78) reportaron el efecto de la suplementación de dietas de roedores con ß-caroteno sobre la inducción de la reductasa del 3-hidroxi-3-metilglutarato. Los mismos autores principales también habían mostrado la existencia de un efecto inibidor en las lesiones preneoplásicas inducidas en hígado de ratas por dietilnitrosamina y 2-acetilaminofluoreno, efecto éste que se correlacionaba directamente con la ingestión de ß-caroteno e inversamente con la concentración de vitamina A en los hepatocitos (79, 80).

La paradoja de que los extensos estudios epidemiológicos realizados por investigadores finlandeses contradigan la anteriormente propalada función protectora del ß-caroteno contra ciertos tipos de cáncer, es algo que ha merecido atención y provocado una propuesta de interpretación por Naves y Moreno (81).

Todavía, la acción del ß-caroteno como posible protector contra los daños causados por el oxígeno singlet al organismo, usando el sistema modelo del alga *Gonyaulax polyedra*, ha sido estudiada por DiMascio et al. (82) y Hollnagel et al. (83), mientras que en otro trabajo se mostró que el ß-caroteno puede ser significativamente más eficiente que la vitamina A en

recuperar células hepáticas preneoplásicas, principalmente por su estímulo a la regeneración de lesiones positivas a la γ-glutamiltranspeptidasa (84).

En otra investigación, Dagli et al. (85), usando el sistema modelo de carcinogénesis resistente del hepatocito, mostraron que las lesiones preneoplásicas fueron significativamente menores en ratas tratadas con ß-caroteno, que en las no tratadas, o tratadas con acetato de retinilo. Esto llevó a los autores a proponer que el ß-caroteno reduce, no solo el aparecimiento de lesiones carcinogénicas en el hígado, sino también la reacción de las células ovales en este modelo experimental.

La porfiria eritropoyética es una condición clínica hereditaria o inducida por algunos xenobióticos, que tiene su origen en defectos fotooxidativos (86). Trabajos de DiMascio y colaboradores vienen colectando evidencias de que los carotenoides acumulados en la sangre de humanos cumplen una función protectora de vital importancia contra la acción destructiva del oxígeno singlet. En ese sentido, el licopeno puede tener función más importante que los demás, visto que éste tiene poder extinguidor (*quencher*) mayor que el de cualquier otro carotenoide y su concentración en el plasma es superior, inclusive a la del ß-caroteno.

Perspectivas y necesidades

Futuramente, el país deberá continuar con los estudios de exploración analítica. Esto es, incrementar el volumen de datos sobre composición de alimentos, tanto los clásicos como los no convencionales. Para tal fin, deberá continuarse a prestar extremo cuidado a las técnicas analíticas, no economizando esfuerzos en la aplicación de los más rigurosos patrones de calidad. Los estudios analíticos y de composición, además de colocar a disposición una base cada vez mayor de alternativas para los estudios agronómicos, químicos, bioquímicos, nutricionales, médicos e industriales, dejarán el saldo positivo de un mayor contingente de técnicos específicamente capacitados en esta área.

En este sentido, espérase que el guía práctico sobre el análisis de carotenoides (16) venga a contribuir en la identificación y corrección de errores importantes que son de común ocurrencia. El libro podrá ser de utilidad no solo para los países latinoamericanos, sino para todo laboratorio que trabaje con carotenoides.

Se prevee expansión de los estudios químicos sobre la degradación de los carotenoides y su influencia en la evolución de aromas y sabores, tanto indeseables como deseables. Esta línea de investigación deberá ser extendida, de sistemas modelo para sistemas reales, evaluándose la posible relevancia biológica y tecnológica de tales transformaciones, inclusive buscando medios de proteger los carotenoides durante los procesos, almacenaje, etc.

Es necesario que los estudios sobre biodisponibilidad de carotenoides evoluyan mediante el uso de técnicas más apropiadas; no con el objetivo de dificultar la investigación, sino para obtener datos que sean más relevantes. Los avances recientes sobre la conversión del ß-caroteno en vitamina A muestran que se trata de un proceso de gran complejidad. La biodisponibilidad puede depender de más de doce factores, sin considerar la influencia de grupos étnicos, estados etarios o estados patológicos. Como ya se sabe que las diversas especies de animales responden de formas diferentes a la utilización de los carotenoides, entiéndese entonces que los estudios deben ser objetivamente canalizados para la especie humana, si lo que se busca es contribuir para la solución de los problemas de salud pública. Aquí, valga decir, las técnicas analíticas tienen importancia fundamental, pues sin una cuantificación adecuada, cualquier conclusión es posible.

Otra necesidad está en profundizar el conocimiento sobre los medios de mejorar la biodisponibilidad, sea obviando las pérdidas debidas a procesos y preparos inadecuados, o justificando la escogencia de las fuentes más apropiadas para cada situación. Este campo se muestra bastante fértil, no solo por lo que el estudio de nuevas fuentes puede traer, sino también por la posibilidad de involucrar las modernas técnicas biotecnológicas en la producción industrial de estos pigmentos y sus derivados. Con relación a ese tema, es interesante tener presente que el fruto amazónico buritĺ es la fuente más rica conocida en ß-caroteno, aún cuando agronómicamente la palma africana tenga rendimiento mayor.

Los temas de investigación que involucran carotenoides diferentes del ß-caroteno y acciones y funciones distintas de la provitamínica A, sin duda, continuarán a llamar mucho la atención. Es temeridad, sin embargo, pensar que ciertas investigaciones con carotenoides puedan ser realizadas sin que se tenga suficiente conocimiento o destreza analítica. El estudio de las funciones y acciones biológicas, por ejemplo, no puede basarse simplemente en el empleo de patrones comerciales, dispensando así cualquier envolvimiento con las técnicas analíticas. Está demostrado que aquellos se degradan con extremada facilidad y, por consiguiente, poseen grados muy variables de pureza. De igual forma, es importante sentir seguridad con los métodos analíticos cuando se estudian los inesperados caminos que el metabolismo o bioconversión de un carotenoide puede tomar después de ser incorporado por el tejido animal. Las formas *cis* de carotenoides como el licopeno están siendo cada vez más implicadas en los procesos de absorción y, tal vez, protección, como muestra la literatura mundial. Es menester entonces enfatizar nuevamente que las expectativas de desarrollar nuestra capacidad y credibilidad investigativa, llegando al dominio de la química y bioquímica de los carotenoides, dependerán directamente de la capacidad de manipular y analizar estos compuestos con confianza.

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REFERENCIAS

1. Rodriguez-Amaya DB, Kimura M, Godoy HT, Arima HK. Assessment of provitamin A determination by open column chromatography/visible absorption spectrophotometry. J Chromatogr Sci 1988; 26:624-9.
2. Mercadante AZ, Rodriguez-Amaya DB. Comparison of normal-phase and reversed-phase gravity flow column methods for provitamin A determination. Chromatogr 1989; 28: 249-52.
3. Kimura M, Rodriguez-Amaya DB, Godoy HT. Assessment of the saponification step in the quantitative determination of carotenoids and provitamins A. Food Chem 1991; 35: 187-95.
4. Carvalho PRN, Rodriguez-Amaya DB, Collins CH, Carvalho CRL. Determinação de carotenóides e provitamina A por cromatografia líquida de alta eficiência (CLAE). Coletânea ITAL 1991; 21: 317-28.
5. Mercadante AZ, Rodriguez-Amaya DB. Desempenho da camada delgada versus camada delgada de alta eficiência multidesenvolvimento para triagem de carotenóides. Ciênc Tecnol Aliment 1991; 11: 200-9.
6. Rodriguez-Amaya DB, Tavares CA. Importance of cis-isomer separation in determining provitamin A in tomato and tomato products. Food Chem 1992; 45: 297-302.
7. Carvalho PRN, Collins CH, Rodriguez-Amaya DB. Comparison of provitamin A determination by normal-phase, gravity-flow column chromatography and reverse-phase high performance liquid chromatography. Chromatogr 1992; 33: 133-7.
8. Godoy HT, Rodriguez-Amaya DB. Avaliação das metodologias para a determinação das pró-vitaminas A. Rev Farm Bioquím Univ S Paulo 1993; 29: 17-24.
9. Wilberg VC, Rodriguez-Amaya DB. Quantificação de ß-caroteno e licopeno em tomate e alguns de seus produtos por CLAE. Ciênc Tecnol Aliment 1993; 13: 132-41.
10. Pereira MR, Amaya-Farfan J, Rodriguez-Amaya DB. Avaliação da metodologia analítica para determinação de ß-caroteno em macarrão fortificado. Ciênc Tecnol Aliment 1998; 18: 35-8.
11. Wilberg VC, Rodriguez-Amaya DB. Quantificação dos carotenóides de urucum (Bixa orellana L.) por cromatografia líquida de alta eficiência (CLAE). Rev Bras Cor Natur 1992; 1: 145-52.
12. Rodriguez-Amaya DB. Critical review of provitamin A determination in plant foods. J Micronutr Anal 1989; 5: 191-225.
13. Rodriguez-Amaya DB. Provitamin A determination. Problems and possible solutions. Food Nutr Bull 1990; 12: 246-50.
14. Rodriguez-Amaya DB, Amaya-Farfan J. Estado actual de los métodos analíticos para determinar provitamina A. Arch Latinoamer Nutr 1992; 42: 180-91.
15. Rodriguez-Amaya DB. Carotenoids: Properties and determination. In: Macrae R, Robinson RK, Sadler M, editors. Encyclopaedia of food science, food technology and nutrition, vol. 1. London: Academic Press, 1993: 707-13.
16. Rodriguez-Amaya DB. A guide to carotenoid analysis in foods. Washington, DC: ILSI Press, 1999 (in press).
17. Godoy HT, Rodriguez-Amaya DB, Connor AE, Britton G. Confirmation of the structure of papaya ß-cryptoxanthin monoepoxide. Food Chem 1990; 36: 281-6.
18. Mercadante AZ, Steck A, Rodriguez-Amaya DB, Pfander H, Britton G. Isolation of methyl 9'Z-apo-6'lycopenoate from *Bixa orellana*. Phytochem 1996; 41: 1201-3.
19. Mercadante AZ, Rodriguez-Amaya DB. Britton G. HPLC and mass spectrometric analysis of carotenoid from mango. J Agric Food Chem 1997; 45: 120-6.
20. Mercadante AZ, Steck A, Pfander H. Carotenoids from guava (*Psidium guava L.*). Isolation and structure elucidation. J Agric Food Chem 1999, 47: 145-51.
21. Almeida LB, Penteado MVC, Simpson KL, Britton G, Acemoglu M, Eugster CH. Isolation and characterization of (5R,6S,5'R,8'R)- and (5R,6S,5'R,8'S)-luteochrome from Brazilian sweet potatoes (*Ipomoea batatas* Lam). Helv Chim Acta 1986; 69: 1554-8.
22. Almeida LB, Penteado MVC, Britton G, Acemoglu M, Eugster CH. Isolation and absolute configuration of beta, beta-carotene diepoxide. Helv Chim Acta 1988; 71: 31-2.
23. Mercadante AZ, Britton G, Rodriguez-Amaya DB. Carotenoids from yellow passion fruit (*Passiflora edulis*). J Agric Food Chem 1998; 46: 4102-6.
24. Godoy HT, Rodriguez-Amaya DB. Ocurrence of *cis* isomers of provitamin A in Brazilian fruits. J Agric Food Chem 1994; 42: 1306-13.
25. Godoy HT, Rodriguez-Amaya DB. Ocurrence of *cis*-isomers of provitamins A in Brazilian vegetables. J Agric Food Chem 1998; 46: 3081-6.
26. Trigueiro INS, Penteado MVC. Stereochemical isomer composition and vitamin A value of Brazilian palm oil. Arch Latinoamer Nutr 1996; 46: 334-7.
27. Rodriguez-Amaya DB, Bobbio PA, Bobbio FO. Carotenoid composition and vitamin A value of the Brazilian fruit *Cyphomandra betacea*. Food Chem 1983; 12: 61-5.
28. Padula M, Rodriguez-Amaya DB. Characterisation of the carotenoids and assessment of the vitamin A value of Brasilian guava. Food Chem 1986; 20: 11-9.
29. Penteado MVC, Minazzi RS, Almeida LB. Carotenóides e atividade pró-vitamínica A de folhas de hortaliças consumidas no Norte do Brasil. Rev Bioquím Farm Univ S Paulo 1986; 22: 97-102.
30. Almeida LB, Penteado MVC. Carotenóides com valor pró-vitamínico A da mandioquinha (*Arracacia xanthorrhiza* Bancr.) consumida em São Paulo, Brasil. Rev Bioquím Farm Univ S Paulo 1987; 23: 52-7.
31. Almeida LB, Penteado MVC. Carotenóides com atividade pró-vitamínica A de cenouras (*Daucus carota* L.) comercializadas em São Paulo, Brasil. Rev Bioquím Farm Univ S Paulo 1987; 23: 133-41.
32. Ramos DMR, Rodriguez-Amaya DB. Determination of the vitamin A value of common Brazilian leafy vegetables. J Micronutr Anal 1987; 3: 147-55.
33. Almeida LB, Penteado MVC. Carotenoids and pro-vitamin A value of white-fleshed Brazilian sweet potatoes (*Ipomoea batatas* Lam.). J Food Comp Anal 1988; 1: 341-2.
34. Arima HK, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of commercial Brazilian squashes and pumpkins. J Micronutr Anal 1988; 4: 177-91.
35. Penteado MVC, Almeida LB. Ocorrência de carotenóides em raízes de cinco cultivares de mandioca (*Manihot esculenta* Crantz) do Estado de São Paulo. Rev Bioquím Farm Univ S Paulo 1988; 24: 39-49.
36. Godoy HT, Rodriguez-Amaya DB. Carotenoid composition of commercial mangoes from Brazil. Lebensm Wiss Technol 1989; 22: 100-3.

37. Minazzi-Rodrigues RS, Penteado MVC. Carotenóides com atividade pró-vitamínica A em hortaliças folhosas. Rev Bioquím Farm Univ S Paulo 1989; 25: 39-52.
38. Rodriguez-Amaya DB, Kimura M. Carotenóides e valor de vitamina A em cajá (*Spondias lutea*). Ciênc Tecnol Aliment 1989; 9: 148-62.
39. Arima HK, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of a squash and a pumpkin from Northeastern Brasil. Arch Latinoamer Nutr 1990; 40: 284-92.
40. Mercadante AZ, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of some native Brazilian green leafy vegetables. Int J Food Sci Technol 1990; 25: 213-9.
41. Trujillo-Quijano JA, Rodriguez-Amaya DB, Esteves W, Plonis GF. Carotenoid composition and vitamin A value from four Brazilian palm fruits. Fat Sci Technol 1990; 6: 222-6.
42. Cavalcante ML, Rodriguez-Amaya DB. Carotenoid composition of the tropical fruits *Eugenia uniflora* and *Malpighia glabra*. In: Charalambous G, editor. Food science and human nutrition. Amsterdam: Elsevier Science Publishers, 1992: 643-50.
43. Almeida-Muradian LB, Penteado MVC. Carotenoids and pro-vitamin A value of some Brazilian sweet potato cultivars (*Ipomoea batatas* Lam.). Rev Bioquím Farm Univ S Paulo 1992; 28: 145-54.
44. Trigueiro INS, Penteado MVC. Teorēs de alfa e beta caroteno e atividade pró-vitamínica a de óleos de dendê brasileiros. Rev Bioquím Farm Univ S Paulo 1992; 28: 61-74.
45. Godoy HT, Rodriguez-Amaya DB. Buriti (*Mauritia vinifera*, Mart.), a very rich source of provitamin A. Arq Biol Tecnol 1995; 38: 109-20.
46. Godoy HT, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of Brazilian loquat (*Eriobotrya japonica*, Lind). Arch Latinoamer Nutr 1995; 45: 336-9.
47. Penteado MVC, Minazzi RS, Almeida, LB. Carotenóides e atividade provitamínica A de folhas de hortaliças consumidas no Norte do Brasil. Rev Bioquím Farm Univ S Paulo 1996; 22: 97-102.
48. Kimura M, Rodriguez-Amaya DB, Yokoyama SM. Cultivar differences and geographic effects on the carotenoid composition and vitamin A value of papaya. Leben Wissen Technol 1991; 24: 415-8.
49. Cecchi HM, Rodriguez-Amaya DB. Carotenoid composition and vitamin A value of fresh and pasteurized cashew-apple (*Anacardium occidentale* L.) juice. J Food Sci 1981; 46: 147-9.
50. Cecchi HM, Rodriguez-Amaya DB. Carotenóides e valor de vitamina A em suco de maracujá processado. Ciênc Cult 1981; 33: 72-6.
51. Padula M, Rodriguez-Amaya DB. Comparison of the carotenoid composition and general properties of the processed juice of guava cultivar IAC-4 and commercial juices. Ciênc Tecnol Aliment 1983; 3: 109-16.
52. Hiani PA, Penteado MVC. Mudança na composição de carotenóides da bocaiúva (*Acrocomia mokayayba* Barb. Rodr.) com a estocagem em congelador a -20°C. Rev Bioquím Farm Univ S Paulo 1989; 25: 169-76.
53. Tavares CA, Rodriguez-Amaya DB. Carotenoid composition of Brazilian tomatoes and tomato products. Lebens Wissen Tecnol 1994; 27: 219-24.
54. Wilberg VC, Rodriguez-Amaya DB. Quantification of major carotenoids of fresh and processed guava, mango and papaya. Lebens Wissen Technol 1995; 28: 474-80.
55. Agostini TS, Cecchi HM, Godoy HT. Composição de carotenóides no marolo *in natura* e em produtos de preparo caseiro. Ciênc Tecnol Aliment 1996; 16: 67-71.
56. Almeida-Muradian LB, Popp V, Farias MP. Provitamin A activity of Brazilian carrots: Leaves and roots, raw and cooked and their chemical composition. Ciênc Tecnol Aliment 1997; 17: 120-4.
57. Bianchini R, Penteado MVC. Carotenóides de pimentões amarelos (*Capsicum annum* L.). Caracterização e verificação de mudanças com o cozimento. Ciênc Tecnol Aliment 1998; 18: 283-8.
58. Godoy HT, Rodriguez-Amaya DB. Composição de carotenóides em nectarina (*Prunus persica*) brasileira. Rev Inst Adolfo Lutz 1998; 57: 73-9.
59. Pereira MR, Amaya-Farfan J, Rodriguez-Amaya DB. ß-caroteno content of Brazilian fortified pasta. Food Control 1999; 10: 81-5.
60. Mercadante AZ, Rodriguez-Amaya DB. Carotenoid composition of a leafy vegetable in relation to some agricultural variables. J Agric Food Chem 1991; 39: 1094-7.
61. Padula M, Rodriguez-Amaya DB. Changes in individual carotenoids and vitamin C on processing and storage of guava juice. Acta Alim 1987; 16: 209-16.
62. Godoy HT, Rodriguez-Amaya DB. Changes in individual carotenoids on processing and storage of mangoes (*Mangifera indica*) slices and puree. Int J Food Sci Technol 1987; 22: 451-60.
63. Godoy HT, Rodriguez-Amaya DB. Comportamento dos carotenóides de purée de mamão (*Carica papaya*) sob processamento e estocagem. Ciênc Tecnol Aliment 1991; 11: 210-20.
64. Rodriguez-Amaya DB. Stability of carotenoids during storage of foods. In: Charalambous G, editor. Shelf-life studies of foods and beverages. Chemical, biological, physical and nutritional aspects. Amsterdam: Elsevier Science Publishers, Amsterdam, 1993: 591-628.
65. Trigueiro INS, Penteado MVC. Mudanças nos teores de alfa e beta-caroteno em óleos de dendê durante o armazenamento à temperatura ambiente (26 C). Bol CEPPA 1993; 11: 1-10.
66. Ramos DMR, Rodriguez-Amaya DB. Avaliação das perdas de carotenóides e valor de vitamina A durante desidratação e liofilização industrial de espinafre. Arq Biol Tecnol 1993; 36: 83-94.
67. Mercadante AZ, Rodriguez-Amaya DB. Effects of ripening, cultivar differences and processing on the carotenoid composition of mango. J Agric Food Chem 1998; 46: 128-30.
68. Costa MAL, Penteado MVC. Alterações decorrentes de dois tipos de cozimento sobre os teores de carotenóides provitamínicos A em escarolas (Chicorium endivia L.). Rev Bioquím Farm Univ S Paulo 1996; 32: 95-100.
69. Sant'Anna ZM, Penteado, MVC. Alterações dos carotenóides decorrentes do cozimento convencional e em forno microondas de três hortaliças. Rev Bioquím Farm Univ S Paulo 1996; 32: 53-8.
70. Almeida-Muradian LB, Fiorini F, Penteado MVC. Provitamin A evaluation of external and internal leaves of cabbage (*Brassica oleracea L.* var capitata L.). Ciênc Tecnol Aliment 1995; 15: 108-11.

71. Rodriguez-Amaya DB. Assessment of the provitamin A contents of foods. The Brazilian experience. J Food Comp Anal 1996; 9: 196-230.
72. Rodriguez-Amaya DB. Nature and distribution of carotenoids in foods. In: Charalambous G, editor. Shelf-life studies of foods and beverages. Chemical, biological, physical and nutritional aspects. Amsterdam: Elsevier Science Publishers, 1993: 547-89.
73. Rodriguez-Amaya DB. Carotenoids and food preparation: The retention of provitamin A carotenoids in prepared, processed and stored foods. Arlington: OMNI, 1997.
74. Yuyama LKO, Fávaro RMD, Yuyama K, Vannucchi H. Biovailability of vitamin A from peach palm (*Bactris gasipaes* HBK) and from mango (*Mangifera indica L*) in rats. Nutr Res 1991; 11: 1167-75.
75. Dutra de Oliveira JE, Fávaro RMD, Junqueira-Franco MVM, Carvalho CG, Jordão Jr AA, Vannucchi H. Effect of heat treatment on the biological value of ß-carotene added to soybean cooking oil in rats. Int J Food Sci Nutr 1998; 49: 205-10.
76. Mariath JGR, Lima MCC, Santos LMP. Vitamin A activity of buriti (*Mauritia vinifera* Mart.) and its effectiveness in the prevention of xerophthalmia. Am J Clin Nutr 1989; 49: 849-53.
77. Dutra de Oliveira JE, Fávaro RMD, Leonardo IR, Jordão AAJr, Vannucchi H. Absorption by humans of ß-carotene from fortified soybean oil added to rice: Effect of heat treatment. J Am Coll Nutr 1998; 17: 1-5.
78. Moreno FS, Rossiello MR, Manjeshwar S, Nath R, Rao PM, Rayalakshmi S, Sarma DSR. Effect of ß-carotene on the expression of 3-hydroxy-3-methylglutaryl coenzyme-A reductase in rat liver. Cancer Let 1995; 96: 201-8.
79. Moreno FS, Rizzi MBSL, Dagli MLZ, Penteado MVC. Inhibitory effects of beta-carotene on preneoplastic lesions induced in wistar rats by the resistant hepatocyte model. Carcinogenesis 1991; 12: 1817-22.
80. Moreno FS, Wu TS, Penteado MVC, Rizzi MBS, Jordão AA, Almeida-Muradian LB, Dagli MLZ A comparison of beta-carotene and vitamin-A effects on a hepatocarcinogenesis model. Int J Vitam Nutr Res 1995; 65: 87-94.
81. Naves MMV, Moreno FS. Beta-carotene and cancer chemoprevention. From epidemiological associations to cellular mechanisms of action. Nutr Res 1998; 18:1807-24.
82. DiMascio P, Hollnagel HC, Speranca M, Colepicolo P. Diurnal rhythm of beta-carotene in photosynthetic alga *Gonyaulax-polyedra*. Biol Chem 1995; 376: 297-301.
83. Hollnagel HC, DiMascio P, Asano CS, Okamoto OK, Stringher CG, Oliveira MC, Colepicolo P. The effect of light on the biosynthesis of beta-carotene and superoxide dismutase activity in the photosynthetic alga *Gonyaulax polyedra*. Braz J Med Biol Res 1996; 29: 105-10.
84. Rizzi MBL, Dagli MLZ, Jordão AA, Penteado MVC, Moreno FS. Beta-carotene inhibts persistent and stimulates remodeling gamma GT-positive preneoplastic lesions during early promotion of hepatocarcinogenesis. Int J Vit Nutr Res 1997; 67: 415-22.
85. Dagli MLZ, Guerra JL, Sinhorini IL, Wu TS, Rizzi MBSL, Penteado MVC, Moreno FS. Beta-carotene reduces the ductular (oval) cell reaction in the liver of Wistar rats submitted to the resistant hepatocyte model of carcinogenesis. Pathol 1998; 30: 259-66.
86. DiMascio P, Medeiros MHG, Bechara EJH, Catalani LH. Singlet molecular oxygen: Generation, reactivity, identification and biological effects. Ciênc Cult 1995; 47: 297-311.

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Necesidades de investigación sobre carotenoides en la República Argentina

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RESUMEN. En la Argentina la información con respecto a la ingesta de nutrientes de la población es escasa, debido a que no se hallan instrumentadas encuestas nutricionales de carácter nacional ni datos actualizados de composición de alimentos. Con respecto a la deficiencia de vitamina A, la Argentina no muestra zonas con sintomatología clínica. Sin embargo, el análisis de las hojas de balance de alimentos y de unas pocas encuestas, que se han realizado en ciertas regiones o en grupos puntuales de la población, indican que existen problemas nutricionales. Los resultados de las autoencuestas llevadas a cabo sistemáticamente por los alumnos de Nutrición de varias Universidades Nacionales han evidenciado cifras indicativas de riesgo con respecto a vitamina A; esos resultados, en algunos casos, se han confirmado con estudios bioquímicos, y son debidos, fundamentalmente, a hábitos alimentarios. Es probable que estos problemas de hábitos alimentarios se agraven en la población con necesidades básicas insatisfechas, cuya prevalencia es elevada en muchas Provincias, así como en las zonas periféricas de los grandes centros urbanos, densamente poblados, principalmente Buenos Aires, Córdoba y Rosario. La información presentada en este trabajo demuestra que se necesitaría contar con datos de encuestas nacionales y de composición de nuestros alimentos, tanto de vitamina A preformada como de carotenoides con y sin actividad provitamínica A, así como evaluar los efectos de la post-cosecha, comercialización y procesos culinarios. Por todo ello y, dado que la última edición de la Tabla de Composición Química de los Alimentos de Argentina, fue producida en 1945, es imperativo emprender a la brevedad los estudios relacionados con la problemática de los carotenoides.
Palabras clave: Carotenoides, composición de alimentos, estado nutricional.

SUMMARY. Investigation needs regarding carotenoid composition in Argentine foods. There are no national dietary surveys regarding nutrient intake of Argentine population. On the other hand, nutrient content of foods in Argentina, as in other Latin American countries, is unknown because there are no data on food composition. Therefore, nutrient intakes must be calculated using foreign composition tables. In relation to vitamin A nutritional status, high prevalence of low vitamin A intake and low plasma retinol levels have been found in the eighties in several groups of universitary students. In this report, results of a 7-day dietary survey of students attending the course of Nutrition in the Universities of Buenos Aires, Luján and Tucumán are presented. Information was processed in a PC Computer (VAN Program, Lujan University, Argentina) to obtain the mean daily intake of carotenes with and without provitamin A activity, according to the German Food Composition Tables. The results showed that provitamin A carotenes provided between 40 and 82% of the vitamin A recommended allowances and that about 20% of the population had total carotene intake lower than 4 mg/day. These results are in agreement with other dietary surveys carried out in students in previous years and are a consequence of some characteristic feeding habits of the Argentine population. In order to obtain more reasonable results regarding actual intakes in our population, study of the composition of national foods would be imperative.
Key words: Carotenoids, food composition, nutritional status

INTRODUCCION

En la Argentina la información existente con respecto a la ingesta de nutrientes de la población es escasa, debido a que no se hallan instrumentadas encuestas nutricionales de carácter nacional. La información disponible proviene del análisis de las hojas de balance de alimentos (1,2) y de unas pocas encuestas que se han realizado en ciertas regiones o en grupos puntuales de la población (3-7).

Entre estas últimas se incluyen las autoencuestas llevadas a cabo por los alumnos que cursan Nutrición en varias Universidades Nacionales, cuyos resultados han sido parcialmente publicados en algunos casos (4-7). Aún cuando estas encuestas están acotadas a cierto espectro de la población, jóvenes de clase media, de entre 20 a 25 años, en general brindan información de tipo sistemático.

A partir de los datos que aportan las fuentes mencionadas, se ha podido apreciar que uno de los nutrientes cuyas ingestas presentan problemas de insuficiencia es la de vitamina A. Los resultados de las autoencuestas mencionadas demuestran que existe un porcentaje de la población estudiantil universitaria que presenta ingestas inferiores a las recomendadas, tanto se tengan en cuenta las cifras aconsejadas por NRC (8) como por FAO (9); los resultados, que se repiten sistemáticamente, han

sido confirmados en algunos casos, mediante indicadores bioquímicos (4, 5). Todo ésto permite inferir que en nuestro país existe un porcentaje de la población que presenta una deficiencia marginal y, en consecuencia, subclínica de vitamina A, atribuible, fundamentalmente, a los hábitos alimentarios.

Por otra parte, para el análisis de encuestas nutricionales se requiere contar con datos confiables de composición de alimentos (10). En ese aspecto, la última edición de La Tabla de Composición Química de los Alimentos de Argentina, fue producida en 1945 por el Instituto Nacional de Nutrición, mediante un programa de composición de alimentos específicamente estructurado para elaborar tablas. En esa edición, precisamente, se incorporó la primera tabla de contenido vitamínico de alimentos vegetales. Si bien los valores de carotenos fueron obtenidos con la metodología recomendada en la época, partición en solventes apropiados, la técnica es obsoleta y tampoco se ha encontrado la documentación sobre la forma en que fueron convertidos en potencia vitamínica. El Instituto fue posteriormente desactivado y no existió continuidad en la producción de datos. En consecuencia, la evaluación de las ingestas de nutrientes de nuestras encuestas, debió realizarse utilizando los datos de los alimentos equivalentes de tablas extranjeras.

En este trabajo se han analizado las autoencuestas llevadas a cabo en las universidades nacionales de Buenos Aires, Luján y Tucumán, con el objetivo de evaluar la contribución de la ingesta de carotenoides provitamina A para cubrir los requerimientos de la vitamina, como así también evaluar el consumo de carotenos, sean activos o inactivos, por ser considerados uno de los nutrientes protectores, por sus propiedades antioxidantes.

MATERIALES Y METODOS

Se analizaron las autoencuestas efectuadas por la población estudiantil de las siguientes casas de estudio: Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Nutrición, estudiantes del segundo semestre de 1998.

Universidad Nacional de Tucumán, Facultad de Farmacia y Bioquímica, estudiantes del segundo semestre de 1998.

Universidad Nacional de Luján, estudiantes de la Carrera de Ingeniería en Alimentos que cursaron la asignatura Nutrición durante los años 1985-98.

Las autoencuestas dietéticas forman parte, en todos los casos, de las actividades prácticas del curso de Nutrición de las respectivas carreras. Los alumnos llevan a cabo la autoencuesta durante 7 días consecutivos, mediante el método de registro del consumo de alimentos. El personal docente les explica, previamente, los objetivos del trabajo, recalcando la necesidad de registrar lo más exactamente posible el peso de todos los alimentos que ingieren. Para facilitar la tarea, se les dan los pesos de las medidas caseras y de las porciones comestibles más comunes. Una vez finalizada la encuesta, los alumnos deben calcular el consumo de cada alimento, expresado en gramos/día, y la correspondiente ingesta de nutrientes promedio diaria. Del total de las encuestas se procesaron las que se ajustaron a las pautas exigidas para su confiabilidad (Buenos Aires: 257; Luján: 216; Tucumán: 58).

Para el análisis y/o revisión de las encuestas se utiliza un programa computarizado desarrollado por docentes de los Departamentos de Tecnología y Ciencias Básicas de la Universidad Nacional de Luján (11). Los datos de composición de carotenos incorporados a las bases de datos del programa, tanto los con actividad como los sin actividad de vitamina A, se tomaron de las Tablas Alemanas de Souci-Fachmann-Kraut (12). De cada encuesta se obtuvieron los siguientes datos promedio/diarios: (a) ingesta de carotenos totales con actividad provitamínica A, (b) ingesta de ß-caroteno, (c) ingesta de carotenoides sin actividad de vitamina A, y (d) Eq. Retinol, calculados mediante los siguientes factores de conversión (9):

Eq. Retinol = µg ß-caroteno/6 + µg de otros carotenoides provitamina A/12

RESULTADOS

En La Tabla se resumen los resultados promedio diarios, los desvíos estándar y los valores extremos, de las ingestas observadas de carotenos con actividad provitamínica A, de equivalentes retinol aportados por dichos carotenos y de carotenoides sin actividad de provitamina A; además, los datos de cada población estudiantil en su conjunto se han desglosados en las respectivas subpoblaciones de mujeres y varones. Como puede observarse, las ingestas promedio diarias, tanto en la población total, como en los subgrupos de las tres poblaciones estudiantiles, no son apreciablemente diferentes; sólo en la población tucumana los valores totales de carotenos activos son algo menores. Pero, en todos los casos, tanto los desvíos estándar como los rangos de consumo promedio diario de todos los grupos, denotan una variabilidad de ingestas extremadamente amplia. En cuanto a los carotenoides sin actividad provitamínica A, es de destacar que en todos los grupos hay encuestas que no registran consumo alguno.

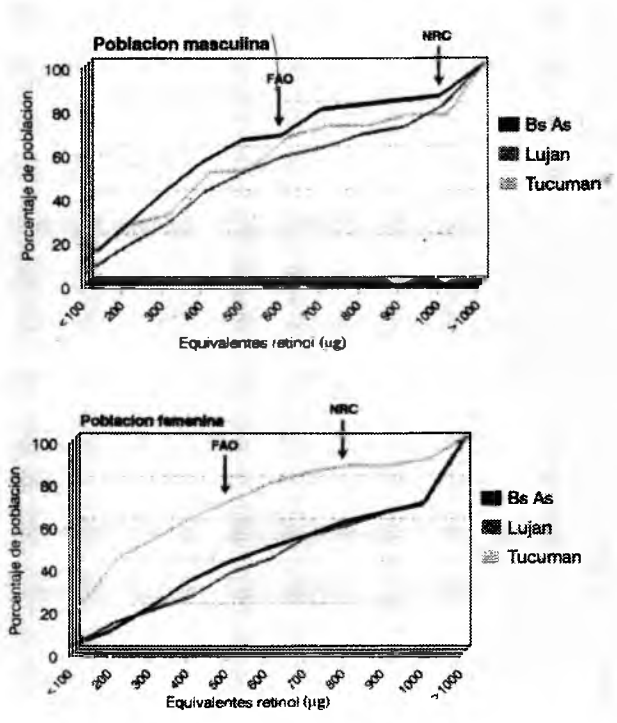
En las Figuras 1a y 1b se grafican, en términos de frecuencia acumulada, los porcentajes de la población femenina y masculina de los tres grupos estudiantiles, en función de la ingesta de Equivalentes de retinol aportada por los carotenoides. En los gráficos, se indican con una flecha los valores de ingestas de vitamina A recomendadas por FAO y NRC, que son, respectivamente: 500 µg y 800 µg de Equivalentes de retinol, para mujeres; 600 µg y 1000 µg de Equivalentes de retinol, para varones. En las poblaciones masculinas puede observarse que alrededor de un 60%, según FAO, y de aproximadamente, un 80%, según NRC, no alcanza a cubrir los requerimientos de vitamina A con los aportes de carotenos activos. En las poblaciones femeninas, en cambio, la distribución es diferente: el porcentaje de las estudiantes de Buenos Aires y Luján que no cubren el requerimiento es de sólo 40%, según FAO, y 58%, según NRC, mientras que en las de Tucumán el porcentaje asciende notoriamente al 65% y 82%, respectivamente.

TABLA 1
Ingesta promedio diaria de carotenos provitamina A, su aporte en equivalentes de retinol y carotenos sin actividad provitamínica A, de las poblaciones estudiantiles

	Número de alumnos	Carotenos activos* (μg)	Eq. Retinol* (μg)	Carotenos* No provit. A (μg)
Buenos Aires				
Población total	257	5503±5239 (100-34440)	710±652 (17-4393)	4150±4494 (0-34083)
Mujeres	182	6176±5501 (409-34440)	791±675 (58-4393)	4294±4554 (0-34083)
Varones	75	3851±4090 (100-23742)	513±543 (17-3375)	3905±4422 (0-29033)
Luján				
Población total	216	5266±4530 (179-26690)	729±627 (19-3963)	4478±4082 (0-28151)
Mujeres	81	6091±4858 (180-26690)	824±674 (19-3963)	4160±3803 (0-17143)
Varones	135	4741±4273 (227-21122)	671±592 (27-3029)	4669±4244 (0-28151)
Tucumán				
Población total	58	3947±4639 (45-22549)	561±671 (7-3205)	4918±5937 (0-37575)
Mujeres	38	3652±5139 (45-22594)	518±744 (7-3205)	4871±6834 (0-37575)
Varones	20	4507±3709 (383-13467)	639±537 (60-1815)	5004±4078 (0-16766)

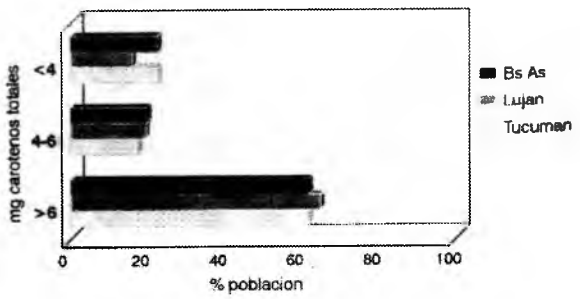
*Promedios ± DE (rangos entre paréntesis)

FIGURA 1
Porcentajes de las poblaciones estudiantiles en función de la ingesta de equivalentes de retinol aportados por los carotenos (en términos de frecuencia acumulada)



En la Figura 2 se graficaron los porcentajes de las poblaciones estudiantiles cuyas ingestas totales de carotenoides, tengan o no actividad, están por debajo de 4 mg, entre 4 y 6 o por sobre 6 mg diarios. Entre el 15-20% de las poblaciones estudiadas consumen menos de los 4 mg, mientras que alrededor del 60% superan los 6 mg.

FIGURA 2
Distribución de las problaciones estudiantiles según rangos de ingesta de carotenos totales



DISCUSION

Los carotenos son pigmentos naturales, liposolubles, presentes en vegetales verdes y amarillo rojizos. De los más de

600 pigmentos que se han identificado, sólo son vitámeros provitamina A aquellos que tienen un anillo ß-ionona en sus extremos. Los carotenos que presentan estas características, pueden ser transformados en retinal por acción de una deshidrogenasa especifica, presente en la mucosa intestinal, y luego en retinol, por acción de una dehidrogenasa. Al igual que el procedente de los alimentos animales, el retinol es absorbido, esterificado y vehiculizado por vía linfática. El ß-caroteno, que en sus dos extremos tiene anillos ß-ionona, es el que presente máxima actividad provitamínica A, ya que, teóricamente, puede originar 2 moléculas de retinol. Sin embargo, por ser muy hidrofóbicos, la absorción de los carotenos es también baja (40-50%), sobre todo en alimentos crudos y cuando el contenido de los lípidos de la dieta es bajo. En consecuencia, la actividad provitamínica A de los carotenos es variable, y se considera que 6 µg ß-caroteno aportan sólo 1 µg retinol, mientras que con los restantes carotenoides con actividad provitamínica A la equivalencia es de 12 µg para obtener 1 µg retinol.

Puesto que la conversión de los carotenos en retinol se efectúa solamente en el epitelio intestinal, cuando la ingesta de carotenos es muy elevada, los que no han sido transformados en retinol en la mucosa intestinal, son absorbidos inalterados unidos a las lipoproteínas, y se depositan en los lípidos de piel y mucosas a las que confieren un típico color amarillento, constituyendo la hipercarotenosis.

La carencia de vitamina A es una de las deficiencias de micronutrientes más extendida en el mundo entero (9,13). En las dietas de los países desarrollados, en general, alrededor del 50% del requerimiento de vitamina A es aportado por los alimentos de origen animal (leche entera, manteca, crema, queso, hígado, huevo y pescados grasos) y el 50% restante, por carotenoides activos; en cambio, en muchos países en vías de desarrollo, donde el consumo de alimentos de origen animal es escaso, el 80-90% de la ingesta de vitamina A es cubierta por provitaminas A. En este aspecto, se está discutiendo si las necesidades de vitamina A pueden ser cubiertas solamente con alimentos vegetales. Mientras que estudios realizados en Gambia aseguran que pueden hacerlo, investigaciones más recientes, han encontrado que son necesarios entre 13 y 76 µg de ß-caroteno de zanahoria para obtener 1 µg retinol. Esto significa que, seguramente, se está sobreestimando, en general, la actividad provitamínica de todos carotenos activos (14).

En nuestro país la información sobre consumo aparente, obtenida a partir de las Hojas de Balance de alimentos indica que los que constituyen la dieta básica Argentina son: trigo, carne vacuna, azúcar y leche en cuarto lugar, aunque el consumo "per capita" es relativamente bajo (alrededor de 200-250 ml/día de leche sin procesar) (I, 2, 15-17); luego, le siguen: aceite de girasol, papa, queso, carne de pollo, visceras vacunas, manzana, mandarina, banana, naranja, durazno, tomate, zapallo y zanahoria. La disponibilidad de hortalizas es baja, con un consumo inferior a 50 kg anuales por habitante, de los cuales el 40% corresponde al tomate. Las hojas de balance no registran gran parte de los datos referidos a verduras de hoja (1,2), y teniendo en cuenta los datos apartados por esas fuentes se puede inferir que la ingesta de vitamina A es inferior a la IR para el adulto (18).

Las escasas encuestas realizadas en algunas zonas del país muestran que, en todos los casos, existe un porcentaje de ingestas insuficientes de vitamina A, que varía según el grupo etáreo y las zonas evaluadas (3-7).

Las autoencuestas estudiantiles anuales muestran que, en este grupo particular de la población, siempre hay un porcentaje de alumnos que no cubren la IR de vitamina A como consecuencia del bajo consumo de leche y también de vegetales. Estas características de los hábitos alimentarios de una cierta franja de la población estudiada quedan evidenciadas a partir de los datos del patrón alimentario, con ingestas promedio de lácteos inferiores a 200 g/día (16,17). En consecuencia, los rangos inferiores de consumo de carotenoides activos que se observan en todas las poblaciones estudiantiles son significativamente bajos y a veces cero. Estos resultados, en algunos casos se han confirmado con estudios bioquímicos, evidenciando en la población estudiantil femenina de la década del '80 un porcentaje alarmante de estudiantes con cifras de retinol plasmático indicativas de riesgo (4, 5).

En la década del '90, el análisis de las autoencuestas de los estudiantes varones de la UBA, reveló un consumo promedio de carotenos de 2570 µg/día, con un rango de 0 a 9726 µg/día; 80.5% de los alumnos presentaron ingestas inferiores a las aconsejadas de 4 mg/día y sólo en el 11% superiores a 6 mg/día. Se debe hacer notar que, en este caso, las Tablas de Composición de Alimentos utilizadas para realizar el análisis de las autoencuestas fueron las del INCAP (19).

No obstante, parecería existir una tendencia a mejorar la situación nutricional con respecto a esta vitamina. Desde hace algunas décadas, las grandes empresas lácteas que distribuyen su producción a nivel nacional fortifican la leche con vitamina A, tanto la descremada como la entera. Además, los indicadores económicos señalan que a partir de 1990 se viene produciendo un incremento muy importante en el consumo interno de ciertos productos lácteos como yogurt, leches cultivadas y quesos untables. Los datos del Informe Estadístico sobre "Leche y Productos lácteos" (15) señalan una disponibilidad total de leche en los últimos años (1990-1996) que equivale a 600 mL/habitante/día; sin embargo, esa cifra incluye la utilizada para elaborar productos lácteos, y los datos de consumo de leche fluída y yogurt reflejan resultados similares a los de la década anterior.

En cuanto a los carotenoides, que no son estrictamente nutrientes esenciales, además de su importancia como precursores de vitamina A, juegan un papel importante como agentes antioxidantes, protectores de la acción tóxica de los radicales libres. Esta función es independiente de su actividad provitamínica A y radica en la capacidad de la molécula de inactivar el singlete de oxígeno y neutralizar peróxidos lipidicos. Los estudios epidemiológicos de las últimas décadas los

incluyen entre los nutrientes protectores, con una acción anticancerígena. Estudios experimentales han demostrado que el antioxidante más potente es el licopeno, muy abundante en tomate. Por ello, se ha sugerido que una ingesta de carotenos entre 4000 y 6000 µg podría tener efectos beneficiosos en cuanto a prevención de ciertos tipos de cáncer (20, 21) y, quizás de otros trastornos derivados de los efectos nocivos de los radicales libres. El cáncer es la segunda causa de muerte en nuestro país después de las enfermedades cardio vasculares (22).

Si bien aproximadamente el 80% de la población estudiada cubre adecuadamente las cifras aconsejadas sobre consumo de carotenoides hay alrededor de un 20% que no alcanza a consumir los 4 mg sugeridos, a consecuencia de sus hábitos alimentarios. De allí que debería intensificarse la educación nutricional para modificar esos hábitos promoviendo el consumo adecuado de frutas y verduras por sus efectos beneficiosos para la salud. Es bastante frecuente encontrar hábitos alimentarios con esas características en la población en general y, en especial, el bajo consumo de frutas y verduras suele observarse en los varones y en los grupos de bajo nivel socio económico (3).

Dado que el contenido de carotenoides en los alimentos varia en función de la especie o variedad, de factores climáticos, agronómicos y del tratamiento postcosecha, es indispensable, contar con datos de composición de los alimentos de producción local cuando se hacen este tipo de estudios. En el caso de los carotenoides, gracias a los avances instrumentales los métodos analíticos permiten identificarlos y cuantificar su contenido.

CONCLUSIONES

La información parcial presentada en este trabajo y los resultados obtenidos de las autoencuestas dietéticas de poblaciones estudiantiles universitarias demuestra que en nuestro país existe un problema nutricional con relación a vitamina A y a ingestas de carotenoides, debido fundamentalmente a los hábitos alimentarios. Se necesitaría contar con datos de encuestas nacionales y de composición de vitamina A de nuestros alimentos, tanto de vitamina A preformada como de carotenoides, para evaluar con mayor precisión la situación nutricional de toda la población.

REFERENCIAS

1. Britos S. Qué es una hoja de balance de alimentos?. Boletín CESNI 1987;1:32-3.
2. Britos S. Vigilancia y canastas alimentarias. Aspectos críticos y progresos. Boletín CESNI 1991;9:37-41.
3. Instituto de Ciencias de la Nutrición del NOA. Memoria Anual 1975. Secretaria de Estado de Salud Pública de la Nación. Universidad Nacional de Salta, 1975.
4. Sanahuja JC, Río ME, de Portela MLPM, Slobodianik N, de Ferrer PR, Friedman SM, Zago L. Estado nutricional con respecto a vitamina A en una población universitaria de Buenos-Aires. Medicina 1985;45: 525-8.
5. Boyer P, de Portela MLPM, Río ME, Sanahuja JC. Evaluación del estado nutricional de una población estudiantil. Medicina 1987;47: 51-6.
6. Rovirosa A, Ribonetto C, del Cerro A, de Portela ML, Río ME. Ingesta de grasas y aceites en una población estudiantil universitaria de Buenos Aires. Arch Latinoamer Nutr 1992;43: 389-94.
7. Rovirosa A, Dupraz H, de Portela ML, Río ME. Ingesta de nutrientes en una población estudiantil masculina de la Universidad de Buenos Aires. Rev Farm (Buenos Aires, Argentina) 1993;133: 53-61.
8. National Academy of Sciences. Recommended Dietary Allowances, 10th. Ed. Washington DC: National Academy Press, 1989.
9. FAO/WHO. Requirements of Vitamin A, Iron, Folate and Vitamin B12. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Series, No 23. Rome: FAO, 1988.
10. Closa SJ, de Portela MLPM, Sambucetti ME, Longo E, Schor I, Carmuega E. Informe de Argentina - Informe sobre estado actual, interés y limitaciones existentes con referencia a "Tabla de Composición de Alimentos en la República Argentina". Arch Latinoamer Nutr 1987;37: 694- 701.
11. Closa SJ, Oloriz M, Marchesich C. Programa electrónico de vigilancia alimentaria nutricional (VAN-UNLu) Abs. 113. IX Congreso Latinoamericano de Nutrición. San Juan (Puerto Rico), Sep. 22-26, 1991.
12. Souci S, Fachmann W, Kraut H. Die Zusammensetzung Der Lebensmittel NahrwertTabellen. Wissenschaftliche Verlagsesellschaft MBH Stuttgart, 1979.
13. Bengoa JM, Torun B, Behar M, Scrimshaw NS. Metas nutricionales y guias de alimentación para América Latina: Bases para su desarrollo. Arch Latinoamer Nutr 1988; 38: 373.
14. West K. Plant sources of vitamin A have a lesser efficacy than preformed vitamin A in the combat against hypovitaminosis A. Debate. 16th Intemational Congress of Nutrition, Montreal (Canadá), 1997.
15. Labriola S, Schaller A. Informe estadístico de leche y productos lácteos,1996. Producción-Elaboración-Consumo-Exportación-Importación. Secretaria de Agricultura, Ganadería, Pesca y Alimentación, Buenos Aires (Argentina), 1997.
16. Pacín A, Martínez E, de Portela ML, Neira MS. Consumo de alimentos en la población de la Universidad Nacional de Luján. Aporte energético y proteico. La Alimentación Latinoamericana 1998;221: 28-36.
17. Pacín A, Martínez E, de Portela ML, Neira MS. Consumo de alimentos e ingesta de algunos nutrientes en la poblacion de la Universidad Nacional de Luján, Argentina. Arch Latinoamer Nutr 1999;49: 31-9.
18. de Portela MLPM. Contenido vitamínico de los alimentos y su relación con el estado nutricional en la Argentina. REVISTA DIAETA (Asociación Argentina de Dietistas y Nutricionistas-Dietistas) 1993; 63: 9-18.
19. Tabla de Composición de Alimentos para uso en América Latina. Instituto de Nutrición de Centro América y Panamá, INCAP e ICNND; Editorial Interamericana, 1969.
20. Lachance P. ß-Carotene and cancer: consumers at risk. I Simposio Argentino de Vitaminas en Medicina, Nutrición y Salud. - Buenos Aires, 11-12 de noviembre, 1992.
21. Kritchevsky SB. ß-Carotene, carotenoids and the prevention of coronary heart disease. J Nutr 1999;129: 5-8.
22. Coniglio RI, Dahinten E, Vidal E, Vasquez LA, Salgueiro AM, Otero JC. Correlación entre variables asociadas con el riesgo para la aterosclerosis coronaria. I. Análisis de una muestra poblacional del Sur Argentino. Acta Bioquim Clin Latinoamer 1992; 26: 35-43.

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Prioridades de investigaciones en el campo de carotenoides en Venezuela

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RESUMEN. La Tabla de Composición de Alimentos (TCA) de Venezuela se encuentra en etapa de revisión para la publicación de su novena edición enmarcada dentro del Proyecto Venezuelan Foods (PVF). El renglón de los pigmentos carotenoides debe ser estudiado casi en su totalidad ya que los valores de que se dispone datan de ediciones de la TCA de 1964 o de 1973 y han sido analizados por un método en columna el cual cuantifica los pigmentos totales sin establecer distinciones entre los diversos carotenoides que pudieran estar presentes y casi todos los valores se refieren al alimento crudo. Recientemente se ha sugerido que los factores de conversión usados para llevar el ß-caroteno y otros carotenos a ER (1/6 y 1/12 respectivamente) no responden a la realidad, así se informa de que la actividad de vitamina A de vegetales frondosos y zanahorias pueden ser de un 23% y en frutas de solo un 50% de lo asumido hasta ahora. Se presentan los porcentajes de adecuación para vitamina A sobre los RDD de Venezuela, de la Encuesta de Consumo de Alimentos, 1998, con los totales de ER sin corregir y corregidos y también con las cifras de disponibilidades para vitamina A suministradas por la Hoja de Balance de Alimento de 1997. Estos resultados varían entre ellos, con lo cual se plantea el interrogante de que si tales adecuaciones han sido sobreestimadas hasta el presente, lo que puede tener influencia en el criterio y decisiones sobre las políticas de fortificación de alimentos y otras acciones pertinentes.
Palabras clave: Tablas de Composición de Alimentos, carotenoides, RDD para Venezuela, equivalentes de retinol.

SUMMARY. Priorities in carotenoid research in Venezuela. The Venezuelan Food Composition Table (VFCT) is being revised for the publication of the ninth edition within the Venezuelan Food Project. The majority of the RE values for plant foods given in the VFCT were taken from previous editions (1964-1973) and with few exceptions, still remains the same in the latest edition of 1994. These values were calculated from the total carotenoid content of food, determined by an open column method with no separation of the various carotenoids that might be present in the food. Recently it has been suggested that the accepted factors to convert ß-carotene and other carotenoids to RE (1/6 and 1/12, respectively) do not reflect the reality and the apparent mean vitamin A activity of leafy vegetables and carrot would be around 23% and fruits about 50% of that assumed until now. Percentages of the Venezuelan RDAs for vitamin A were calculated from vitamin A intake supplied by food consumption surveys, using the conversion factors corrected according to the above mentioned criteria. Percentages of the Venezuelan RDAs were again calculated with the vitamin A availability given by the Food Balance Sheet (1997), using two levels of RE in carrots : 2,800 ER and 850 ER. Results in these approaches differ markedly and indicate that the vitamin A nutrition status may be overestimated. If confirmed, this situation could influence the criteria and decisions in regard to food fortification and other strategies for controlling vitamin A deficiency.
Key words: Food Composition Table, carotenoids, Venezuelan RDAs, retinol equivalents.

INTRODUCCION

La Tabla de Composición de Alimentos (TCA) de Venezuela se encuentra en revisión para la publicación de su Novena Edición a ser editada dentro de la Serie Cuadernos Azules del Instituto Nacional de Nutrición. Los valores de equivalentes de retinol (ER) de la octava edición disponible (1994) (1), se calcularon casi en su totalidad a partir de las cifras expresadas en unidades internacionales, originalmente publicados en la TCA de 1954 (2) y los cuales con pocas excepciones, se han mantenido inalteradas hasta la fecha. Por ejemplo los valores de carotenos en diversas especies de cambures y plátanos cultivados en Venezuela al igual que algunos de sus preparaciones, se determinaron experimentalmente y los resultados dados a conocer en una publicación (3), se incluyeron en la TCA de 1964 (4).

Los cálculos se hicieron tomando en cuenta el factor de conversión estimado de 6:1 para ß-caroteno y de 12:1 para otros carotenoides (5) y la Tabla de Distribución de Actividades de Vitamina A en los Alimentos (1). Así, casi todos estos valores en alimentos de origen vegetal, provienen de análisis efectuados en nuestros laboratorios hace ya más de 40 años y responden al método en columna de Celite - MgO (6), el cual no permite establecer una clara distinción entre los diversos carotenoides que pudieran estar presentes. Otros resultados han sido obtenidos en los años sesenta y setenta por la misma metodología e incorporados en las sucesivas ediciones de la TCA de esa época (7,8) o fueron tomados de otras publicaciones (9,10).

En atención a lo expuesto se plantea la necesidad de revisar y actualizar estos valores como actividad prioritaria dentro del proyecto VenezuelanFoods enmarcado dentro del LatinFoods, que actualmente se adelanta en el país.

Equivalentes de Retinol y la TCA de Venezuela: El contenido de vitamina A por 100 g de parte comestible del alimento aparece en las TCA venezolanas expresado en UI desde 1954 (2) hasta 1964 (4) y ya en la edición siguiente 1973 (7) se cambia a ER. Estos valores se han mantenido inalterados casi en su totalidad como se informa con algunos ejemplos en la Tabla 1, en la cual se aprecia el año en el que aparecen por primera vez y que aun permanecen en la edición de 1994 (1).

Por ejemplo el hígado de res aparece en 1954 con 20.000 UI, cifra esta que se mantiene hasta 1991 expresada como 5600 ER y es elevada a 10.500 ER en la edición de 1994 (1) de acuerdo a resultados obtenidos en otros laboratorios del país. La acelga, la auyama, el mango bocado y la patilla presentan valores de vitamina A en UI de 6.000, 4.959, 3.000 y 400, respectivamente, los cuales aparecen en la TCA de 1954 (2), se repiten en la de 1964 (4) y ya convertidos en ER, se mantienen en la TCA de 1994 (1). La guayaba rosada con un contenido de 6870 UI en la TCA de 1964 (4) exhibe en 1994 (1) 687 ER, es decir el mismo valor. En el caso de la zanahoria el valor inicial publicado también en 1954 (2) era de 8.000 UI, convertido a 800 ER hasta la edición de 1991 (11) y aumentándose a 2.800 ER en la de 1994 (1). Este valor es mayor que el informado en otras TCA y su empleo por ejemplo en la interpretación de encuestas de consumo de alimentos, puede sobrestimar la ingesta de vitamina A en la población objeto de estudio. Por lo anterior y dada la importancia de este renglón y la frecuencia con que aparece en las encuestas de consumo de alimentos, conceptuamos que la nueva edición de la TCA de Venezuela debe tomar en cuenta estos hechos y actualizar el contenido de vitamina A mediante los análisis correspondientes o por selección apropiada de promedios confiables y representativos de la literatura.

TABLA 1
Algunos alimentos cuyos valores de vitamina A no se han modificado desde su publicación inicial en la TCA

Alimento / TCA año	U I / ER
Maíz amarillo / 1950	300/ 30
Maíz tierno / 1950	300 / 30
Leche líquida completa / 1950	150 / 36
Huevos / 1954	1.000 / 240
Hígado de res / 1954	20.000 / 5.600
Yema /1954	3.200 / 768
Acelga / 1954	6.000 / 600
Auyama / 1954	4.950 / 495
Brócoli / 1964	3.500 / 350
Zanahoria / 1954	8.000 / 800
Mango bocado / 1954	3.000 / 300
Patilla / 1954	400 / 40
Guayaba rosada / 1964	6.870 / 687

Equivalentes de Retinol y otras TCA: La Tabla 2 muestra el contenido de vitamina A, expresado en ER, de algunos alimentos vegetales según diversas TCA (1, 2-18). Se aprecia la gran diferencia que existe para un mismo renglón en los ejemplos seleccionados. Esto no debe sorprender, dada las variedades existentes para el mismo producto que pueden encontrarse en el país o región, y también la metodología usada, parte analizada, tratamiento culinario, estado de maduración etc. En el caso de la zanahoria, el valor más elevado corresponde a la TCA de Venezuela (1) con 2.800 ER/ 100 g y el más bajo al señalado por la TCA del Cercano Oriente (18) con 648 ER /100 g. Es de destacar que el valor de 2.800 ER es 78% y 108% mayor que los que corresponden a las TCA

TABLA 2
ER de algunos alimentos vegetales de acuerdo a diversas TCA

Alimento	Venezuela 1994 (1)	Bolivia 1984 (12)	INCAP 1971 (13)	México 1996 (14)	Colombia 1996 (15)	Medpharm 1994 (16)	España 1998 (17)	Near East 1982 (18)
Apio	57	45	-	-	19	-	-	-
Batata	50	57	9	300	50	1430	-	280
Acelga	600	473	293	404	-	588	183	295
Auyama	495	300	305	246	340	128	-	363
Berro	472	491	220	313	240	692	500	333
Espinaca	600	383	392	321	250	781	542	947
Lechosa	151	95	36	33	70	161	98	-
Guayaba	687	-	27	32	40	119	73	28
Mango	300	72	210	245	110	201	-	300
Melón	150	98	117	126	40	-	3	103
Patilla	40	-	23	36	30	87	18	69
Lechuga	162	203	87	44	26	240	29	222
Zapote	45	-	38	18	100	-	-	-
Zanahoria	2800	767	1066	666	700	1570	1346	648

de Europa (16) y de España (17) respectivamente, los cuales se identifican como los valores más altos de las siete TCA consultadas. En este orden de ideas es de interés examinar los resultados de ß-caroteno en zanahorias de diversas procedencias (Tabla 3) compilados por Rodríguez-Amaya (19), y los cuales representan el 85% de la actividad de vitamina A en ese vegetal, de acuerdo con la distribución de actividad de vitamina A en alimentos (5). La actividad de 15% restante provendría de otros carotenoides con una eficiencia mas baja de bioconversión y no podría por lo tanto incrementar significativamente estos valores. Si se calcula el contenido de ß-caroteno que le correspondería al valor de 2.800 ER indicado en la TCA de Venezuela (1), empleando la equivalencia de 1:6, se obtiene la cantidad de 14.280 µg de ß-caroteno por 100 g de parte comestible, en ningún modo comparable con los ilustrados en la Tabla 3.

TABLA 3
Contenido de ß-caroteno en zanahorias de diversas procedencias*

País / año	µg/g	µg/100 g de parte comestible
India / 1995	65	6.500
Malasia / 1991	68	6.800
Taiwan / 1993	54	5.400
Nepal / 1995	43	4.300
Japón / 1986	43	4.300
Finlandia / 1989	76	7.600
Brasil / 1988	34	3.400
Egipto / 1991	63	6.300

*Adaptado de Rodríguez Amaya (19)

Implicaciones en la interpretación de las encuestas alimentarias y en la elaboración de las Hojas de Balance de Alimentos

Escenario 1

La importancia de disponer de datos confiables, en este caso el contenido de vitamina A, se demuestra al interpretar la información relacionada con el consumo de alimentos obtenida en el estudio del impacto del enriquecimiento de la harina de maíz precocida y de trigo en Venezuela, realizado entre Noviembre de 1996 y Mayo de 1998 (20). Como un esfuerzo conjunto entre FUNDACREDESA y UNICEF, este estudio recoge entre una gran variedad de datos, el consumo de alimentos en seis municipios de la zona metropolitana de Caracas, de donde se han seleccionado para este ejemplo solo las hortalizas. La Tabla 4 ilustra específicamente el consumo de vitamina A para los estratos sociales III, IV, V, es decir los de menor poder adquisitivo, aportado por las principales hortalizas adquiridas por las familias y expresado en ER. Para una mejor lectura y no sobrecargar la información, se informa del total de ER sin discriminar lo aportado en particular por cada hortaliza y se ha calculado el porcentaje de adecuación sobre las recomendaciones, para la población venezolana (21). Las cifras sin paréntesis han sido estimadas con la información de la TCA de 1994 (1) la cual indica para la zanahoria un contenido de 2.800 ER por 100 g. En este caso el porcentaje de adecuación sobre las recomendaciones es de 103%, 99% y 84% para los Estratos III, IV y V respectivamente. Por otro lado, las cifras entre paréntesis se calcularon igual pero tomando para la zanahoria un contenido de 850 ER por 100 g, manteniéndose inalterado desde luego, el aporte de las demás hortalizas. Como era de esperarse, se aprecia que ahora los porcentajes de adecuación caen a menos de 50% para cualquiera de los tres Estratos, (cifras entre paréntesis). Este sencillo cálculo, aunque aislado se basa en realidades e ilustra sobre como un dato exageradamente elevado - y en este caso creemos que lo es - puede modificar sustancialmente la apreciación de la adecuación de un nutriente. Por supuesto hay que tomar en cuenta la cifra global entregada por el resto de los alimentos, ya que por tratarse solo de un ejemplo, no se consideró el aporte de vitamina A de los otros alimentos ni tampoco el proveniente de los productos enriquecidos.

TABLA 4
Aporte en ER de las principales hortalizas adquiridas por las familias según Estrato Social. Condiciones de Vida. 1997

Estrato Social	ER		Adecuación, %	
III	828*	(356)**	103*	(44)**
IV	791	(338)	99	(42)
V	673	(282)	84	(35)

Hortalizas: ají, auyama, lechuga, pimentón, tomate, brócoli, zanahoria, otros.
*Calculados tomando un aporte de 2.800 ER/100 g zanahoria
**Calculados tomando un aporte de 850 ER/100 g zanahoria

Escenario 2

Recientemente se ha dado a conocer que los factores de equivalencia aceptados y usados hasta el presente para convertir el ß-caroteno a ER, es decir 1:6, no responden a la realidad (22-24). Así, se informa que la actividad de vitamina A de vegetales frondosos y zanahorias puede ser de un 23% y en frutas de solo un 50% de lo asumido hasta ahora. De acuerdo al razonamiento de De Pee y colaboradores (25), se establece un factor de 5, por el cual habría que corregir el valor de ER derivados de los carotenoides provenientes de alimentos de origen vegetal.

En este marco de referencia, se hace nuevamente alusión a la encuesta que informa sobre los grupos de alimentos adquiridos en los hogares de los Estratos Sociales III, IV, V, dentro del estudio antes mencionado (20). La Tabla 5 puntualiza el consumo de vitamina A en estos Estratos, como ER/

persona/día entregado por los diversos grupos de alimentos que allí se citan. La Tabla 6 recoge esta información ya simplificada y modificada para ser interpretada a la luz de los recientes hallazgos mencionados. Tomando como ejemplo el Estrato III las modificaciones son: a) al total de alimentos de origen vegetal (1.392,5 ER, Tabla 5) se le resta el valor correspondiente a los cereales y a las grasas visibles, toda vez que estos renglones están enriquecidos de acuerdo a sus respectivas Normas COVENIN (26, 27) y en consecuencia no aplica lo del factor de corrección . Este total es ahora 1.139,5 ER (Tabla 6).

TABLA 5
Consumo de vitamina A por Estrato Social.
ER / persona / día

	III	IV	V
Cereales	205,4	238,2	257,9
Tubérculos	85,6	62,4	51,4
Leguminosas	7,8	7,4	8,2
Hortalizas	843,8	732,5	539,8
Frutas	191,5	168,1	121,2
Grasas visibles	47,6	45,6	46,5
Varios	10,8	10,4	9,8
Sub-total	1.392,5	1.255,6	1.034,8
Leche, lácteos, huevos	318,9	284,8	255,4
Carnes y pescados	667,4	607,8	513,5
Total	2.378,8	2.148,2	1.803,7

TABLA 6
Aporte total de ER por alimentos vegetales y animales y adecuación sobre las RDD

Estrato Social	Origen Vegetal	Origen Animal	Total	Adecuación %
III	1139,5 (228)	1239,3	2378,8 (1467,3)	297 (183)
IV	971,8 (194)	1176,4	2148,2 (1370,4)	268 (171)
V	730,4 (146)	1072,9	1803,3 (1218,9)	225 (152)

Valores ( ) representan valores corregidos

b) Los valores a su vez, se añaden a los ER proveniente de los alimentos de origen animal (986,3 ER) para dar un total de 1.239,3 ER, el cual sumado al valor anterior da el gran total de 2378,8 ER (Tabla 5). c) A continuación el valor de ER de los alimentos vegetales se divide entre 5, dando origen a la primera cifra entre paréntesis de la segunda columna de la Tabla 6, la cual sería el valor real a considerar, y que en el caso del Estrato III es de 228 ER. d) Este valor se suma al de los alimentos animales (1.239,3 ER) para originar el total de 1.467,3 que figura entre paréntesis. e) Finalmente se calculan los porcentajes de adecuación sobre los totales sin paréntesis, 297% y sobre los valores corregidos aplicando el factor de 5, con paréntesis, 183%. En definitiva, salta a la vista que la adecuación correspondiente al total de ER consumido por los Estratos III, IV y V, 297%, 268% y 225% respectivamente, se ve sensiblemente disminuida si se acepta el nuevo enfoque propuesto por De Pee y colaboradores (25).

Escenario 3

Para transformar las disponibilidades alimentarias entregadas por las Hojas de Balance de Alimentos (HBA) en disponibilidades de nutrientes, es imprescindible el disponer de los datos sobre composición de alimentos aportados por las TCA. En el caso que nos ocupa, vitamina A, un valor desusadamente elevado de este nutriente para un renglón importante en un grupo determinado de alimentos, puede sobrestimar el aporte de esa vitamina en el grupo. Por ejemplo la zanahoria es responsable por un 48,6% del total aporte de vitamina A en el grupo de las hortalizas (28). Dentro de este escenario, la Tabla 7 ilustra sobre la disponibilidad un g/ persona/día de zanahoria, según la HBA de 1997 (28), el total de ER y el porcentaje de adecuación sobre los Requerimientos (21), el cual es de 159,5%. Es de hacer notar que este valor se calcula dividiendo el total de ER entre el Factor de Pérdidas Detal-Boca del consumidor, estimado para Venezuela por el Centro de Investigaciones Agroalimentarias de la Universidad de Los Andes (28). La segunda mitad de la Tabla 7 muestra similar apreciación pero esta vez tomando en cuenta un contenido de 850 ER/100 g para la zanahoria en vez de 2.800 ER/100 g (1), empleado en el cálculo anterior. Se aprecia que el porcentaje de adecuación para la vitamina A en 1997, disminuiría a 115%.

TABLA 7
Adecuación del consumo de vitamina A de acuerdo a la HBA - 1997 (preliminar) y con diferente contenido de vitamina A en la zanahoria

Zanahoria: Disponibilidad persona/día HBA. 1997	Total ER	Requerimiento (21)	Porcentaje de adecuación
ESCENARIO 1			
19,6 g	1.370,7 (666,3)*	800 ER	159,5
ESCENARIO 2			
19,6 g	988,5 (284,1)**	800 ER	115,0

*Total hortalizas utilizando zanahoria con 2.800 ER/100 g HBA, 1997.

**Total hortalizas utilizando zanahoria con 850 ER/100 g Cálculos propios.

COMENTARIOS FINALES

Estos enfoques son desde luego algo especulativos, pero no dejan de sembrar cierta duda e inquietud en cuanto al aporte de vitamina A proveniente de la diversidad de carotenoides encontrados en los alimentos de origen vegetal, tanto mas cuanto que la biodisponibilidad de los carotenoides y su bioconversión a retinol (vitamina A) es influenciada según De Pee y West (29) por los siguientes factores: tipo o especie de carotenoide, cantidad de carotenoides ingerido en cada comida, unión molecular, matriz en la cual se encuentran incorporados, factores genéticos y estado de nutrición de la persona, modificadores de la absorción, preparación culinaria e interacciones variadas. De confirmarse esta situación, ello implicaría una interpretación modificada en cuanto a la magnitud y efectividad de estos aportes, al mismo tiempo que se destacaría aun mas, la importancia del consumo de alimentos de origen animal y la relevancia del enriquecimiento de alimentos de consumo popular y tradicional con vitamina A en caso de ser factible o aconsejable la aplicación de esta estrategia.

REFERENCIAS

1. Tabla de Composición de Alimentos para Uso Práctico. Revisión 1994. Publicación N° 50. Venezuela: Instituto Nacional de Nutrición.
2. Tabla de Composición de Alimentos para Uso Práctico. Revisión 1954 Publicación N° 17. Venezuela: Instituto Nacional de Nutrición.
3. Jaffé W, Chávez JF, Koifman B. Sobre el valor nutritivo de plátanos y cambures. Arch Venez Nutr 1963; 13: 9 -14.
4. Tabla de Composición de Alimentos para Uso Práctico. Revisión 1964. División de Nutrición. Venezuela: Instituto Nacional de Nutrición.
5. Necesidades de Vitamina A, tiamina, riboflavina y niacina. Informe de un Grupo Mixto FAO/OMS de Expertos Informe N° 41. 1967.
6. Methods of vitamin assay. The Association of Vitamin Chemists, Inc. Intercience Publishers, Inc. p. 45-68. 1947.
7. Tabla de Composición de Alimentos para Uso Práctico. Revisión 1973, Publicación N° 31. Venezuela: Instituto Nacional de Nutrición.
8. Tabla de Composición de Alimentos para Uso Práctico. Revisión 1978. Publicación N° 40. Venezuela: Instituto Nacional de Nutrición.
9. Tabla de Composición de Alimentos para Uso en América Latina. INCAP - ICNND. W. T. Wuleung y M. Flores 1961.
10. Composition of foods: raw, processed, prepared. B. K. Watt and A.L. Merril. Agricultural Handbook N° 8 U S D A. 1963.
11. Tabla de Composición de Alimentos para Uso Práctico. Revisión 1991. Publicación N° 47. Venezuela: Instituto Nacional de Nutrición.
12. Tabla de Composición de Alimentos Bolivianos. Ministerio de Previsión Social y Salud Pública. La Paz. Bolivia. 1984.
13. Valor nutritivo de los alimentos para Centro América y Panamá. INCAP. Guatemala. 1971.
14. Tablas de valor nutritivo de los alimentos de mayor consumo en México. Edición Internacional. Editorial Pax México. 1996.
15. Tabla de Composición de Alimentos Colombianos. I C B F. Séptima Edición. Colombia 1996
16. Food Composition and Nutrition Tables. 5th revised and completed edition. MEDPHARM Scientific Publishers. Stuttgart. 1994.
17. Tabla de Composición de Alimentos Españoles 3ra. Edición. Universidad de Granada. 1998.
18. Food Composition Tables for the Near East. Rome: FAO - USDA, 1982.
19. Rodríguez-Amaya DB. Carotenoids and food preparation: The retention of provitamin A carotenoids in prepared, processed and stored foods. Arlington: OMNI, 1997.
20. Impacto del enriquecimiento de las harinas en niños, jóvenes y adultos de la población venezolana. Ministerio de la Secretaría FUNDACREDESA. Caracas, 1998.
21. Necesidades de energía y nutrientes. Recomendaciones para la población venezolana. Publicación N° 48. INN. Fundación CAVENDES. 1993.
22. De Pee S, West CE, Muhilal, Karyadi D, Hautvast JG. Lack of improvement in vitamin A status with increased consumption of dark - green leafy vegetables. Lancet 1995; 346: 75-81.
23. De Pee S, West CE, Permaesih D, Martuti S, Muhilal, Hautvast JG. Orange fruits is more effective than are dark - green, leafy vegetables in increasing serum concentrations of retinol and ß-carotene in schoolchildren in Indonesia. Am J Clin Nutr 1998; 68: 1058-67.
24. De Pee S, Bloem MW, Gorstein J, Sari M, Satoto, Yep R, Shrimpton R, Muhilal. Reappraisal of the role of vegetables in the vitamin A status of mothers in Central Java, Indonesia. Am J Clin Nutr 1998; 68: 1068-74.
25. Bloem MW, de Pee S, Darnton-Hill I. New issues in developing effective approaches for the prevention and control of vitamin A deficiency. Food Nutr Bull 1998; 19: 137-48.
26. Margarina. Norma COVENIN N° 70-1994. (obligatoria) Venezuela
27. Harina de maíz precocida. Norma COVENIN N° 2135 - 1996 (obligatoria) Venezuela.
28. Hojas de Balance de Alimentos. 1996 - 1997. (Preliminar) Instituto Nacional de Nutrición. Universidad de los Andes. Talleres Gráficos Universitarios, ULA, Mérida, Venezuela, 1998.
29. De Pee S, West CE. Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. Eur J Clin Nutr 1996; 50, Suppl: S38-S53.

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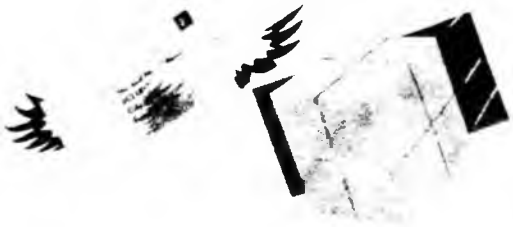

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