Carotenoid Composition in Raw and Cooked Spanish Vegetables†

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An HPLC study of 18 of the fresh vegetables (raw and cooked) most frequently consumed in Spain was done to determine their carotenoid composition. The results are grouped according to the color of the edible portion of each: green, red-orange, or yellowish-white vegetables. β-Carotene and lutein were found to be present in all of the vegetables analyzed except the sweet red pepper, which contains zeaxanthin but not lutein. Lutein and zeaxanthin are present in five of the vegetables analyzed, with the highest concentration of both of these components being found in spinach. In green and yellowish-white vegetables, lutein predominates over β-carotene. Red-orange vegetables show a wider carotenoid profile, in which the lutein levels are surpassed by other carotenoids (e.g., lycopene in tomatoes, α- and β-carotene in carrots). Boiling was not found to alter the carotenoid profile of the samples, but the amounts of carotenoids quantified were higher when compared to those in raw samples.

INTRODUCTION

The carotenoids are dietary components that are not customarily characterized and quantified in the Food Composition Tables most widely utilized in our country (Paul and Southgate, 1978; Instituto de Nutrición, 1980; Souci et al., 1989). These components are ingested in their natural form, present mainly in vegetables and fruits, or as food additives.

At present, the importance of the carotenoids and, thus, of the foods that contain them is mainly based on two factors: their provitamin A activity and their antioxidant capacity. However, the foods we consume also contain abundant amounts of carotenoids with little or no provitamin activity (Katrangi, 1984). This fact, together with evidence gathered by a number of epidemiologic studies dealing with certain types of cancer (Ziegler, 1989; Comstock et al., 1991; Harris et al., 1991), has led to a refocusing of the studies on carotenoid metabolism and its importance in the human organism.

Today it is possible to determine a great number of carotenoids by means of high-performance liquid chromatography (HPLC), the methodology on which we have based our study of the vegetables most commonly consumed by the Spanish population (Malo and del Valle, 1987; Ministerio de Agricultura, Pesca y Alimentación, 1988; Moreiras et al., 1990). Most of these vegetables are cooked prior to consumption; thus, the analyses have been carried out in both raw and cooked state given that the processing of foods leads to variations in their carotenoid concentrations (Bushway and Wilson, 1982; Khachik and Beecher, 1987; Khachik et al., 1991; Varo et al., 1989).

With these analyses, we intend to provide not only data on the provitamin A activity but information on other carotenoids, for a better interpretation of the epidemiologic studies dealing with human diet and health.

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EXPERIMENTAL PROCEDURES

Apparatus. High-performance liquid chromatography was carried out in an ALC/GPC chromatograph (Model 201, Waters Associates, Milford, MA) equipped with a Model 6000 A pump, dual reciprocating piston heads, Model U6K septumless injector, and programmable multiwavelength detector (Model 490E, Waters Associates). The detector signals were recorded on a M730 data module (Waters Associates) and an Omniscribe recorder.

Concentrations of stock standard solutions were determined using an Uvikon 930 spectrophotometer (Kontron Instruments).

Moisture content was determined in a vacuum desiccator (Model Vacuo-temp, Selecta).

Reagents. *all-trans*-β-Carotene, lycopene, *all-trans*-α-carotene, and retinyl palmitate standards were obtained from Sigma Chemical Co. (St. Louis, MO). 15-*cis*-β-Carotene, 9-*cis*-β-carotene, 13-*cis*-β-carotene, phytoene, γ-carotene, canthaxanthin, β-cryptoxanthin, echinenone, lutein, and zeaxanthin were gifts from Hoffmann-La Roche (Basel, Switzerland). β-Apo-8′-carotenal was obtained from Fluka Chemie AG (Switzerland). Stabilized tetrahydrofuran (THF), butylated hydroxytoluene (BHT), and dichloromethane were purchased from Carlo Erba (Spain). Methanol, ethanol, acetonitrile, anhydrous sodium sulfate, and diethyl ether were supplied by Merck (Spain). Potassium hydroxide and magnesium carbonate (anhydrous powder) were supplied by Probus and Scharlau, respectively.

Chromatographic Procedures. Two different columns and two different solvent systems were used for the reversed-phase separation of carotenoids (Olmedilla et al., 1990; Granado et al., 1991). These were as follows.

System I: a 5-μm column Spheri-5-RP-18 or Spheri-5-ODS column (Brownlee Labs, Kontron Analytic), 220 × 4.6 mm, with a guard column of Aquapore ODS type RP-18, 15 × 3.2 mm, 7 μm; solvent, acetonitrile/dichloromethane/methanol (70:20:10); flow rate, 1.8 mL/min; 5–10 μL injected.

System II: a 5-μm column Spheri-5-RP-18 or Spheri-5-ODS column (Brownlee Labs, Kontron Analytic), 220 × 4.6 mm, with a guard column of Aquapore ODS type RP-18, 15 × 3.2 mm, 7 μm; solvent, acetonitrile/methanol (85:15); flow rate, 1.8 mL/min initially, to be increased to 3.5 mL/min at 9.50 min; 5–10 μL injected. This system was used to separate lutein from zeaxanthin.

Carotenoids were detected at 450 nm, retinyl palmitate at 325 nm, phytoene at 286 nm, and phytofluene at 370 nm.

Source of Samples and Their Preparation for Extraction. A 500–1500-g sample of each vegetable was purchased in each of two markets in different points of the city of Madrid and was analyzed immediately. Vegetables were sampled during the months indicated (*) in Figure 1 [the line indicates the months

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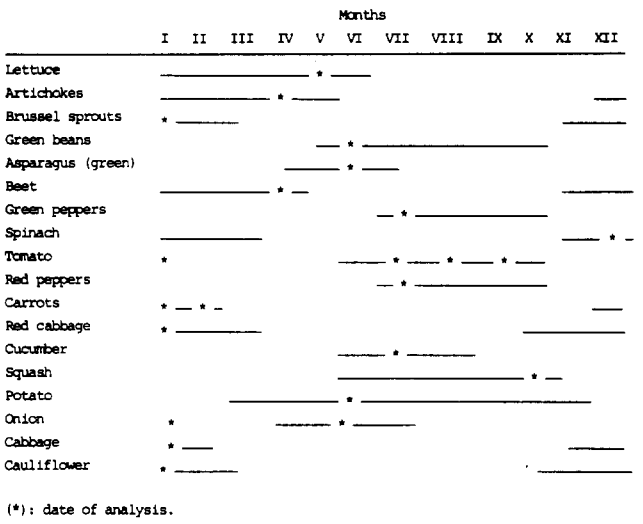

Figure 1. Market season of the vegetables analyzed.

corresponding to the season for each product in Spain, which coincides with their maximum consumption (Malo and del Valle, 1987)].

The nonedible part of the vegetables was eliminated, and large pieces were cut into smaller ones. The produce was rinsed with water, drained and divided into two homogeneous fractions: one for analysis in raw form and the other to be boiled in a covered pot with a minimum of water (500 mL of H_2O per 500-g sample) prior to extraction. The cooking time in each case was that necessary for normal human consumption and is indicated in the tables. The samples were then homogenized in a blender.

The water content was determined in triplicate by drying in a vacuum desiccator at 48 ± 2 °C until constant weight was reached.

The analyses were performed in quadruplicate in aliquots whose size (5–30 g) depended on the intensity of the color of the sample. The extraction method was that reported by Bushway and Wilson (1982) with slight modifications. The aliquots were extracted with 100 mL of THF, stabilized with BHT (0.01%), and placed on a magnetic stirrer (15 min in total darkness, under nitrogen atmosphere), with sodium sulfate and magnesium carbonate (200 and 10% of the weight of the vegetable, respectively), and internal standard was added. The extract was filtered in vacuo, and the solid material was reextracted with THF until the resulting filtrate was colorless. The filtrate was removed on a rotary evaporator at 40 °C to dryness and was then reconstituted with THF (10–25 mL) and injected into chromatographic system I. An aliquot of this extract was evaporated and reconstituted with THF/EtOH (1:3) and injected into chromatographic system II.

Saponification. In cases in which the vegetables were saponified, ethereal solutions of the extracts (approximately 10 mL) were treated with saturated methanolic potassium hydroxide (1 mL) for 30 min under nitrogen atmosphere and protected from light. The solution was separated into a diethyl ether (40 mL) and ether-saturated water (10 mL), and the organic layer was removed, to be washed several times with ether-saturated water (10 mL) until the KOH was completely removed (pH 7.0). The solvent was evaporated and the residue dissolved in the appropriate solvent for chromatographic analyses.

Internal Standard. Echinenone (0.5–2.5 μg/mL) and retinyl palmitate (2.5–6.5 μg/mL) were assayed as internal standards for assessing the possible loss of carotenoids in the samples during the extraction and saponification processes.

Identification and Quantitation. The carotenoids were identified by comparing their retention times with those of authentic standards, by simultaneous injection of standards and sample, and, in some cases, by stop-flow scan at the top of the peak.

They were quantified in a given sample by means of a calibration curve that included all of the carotenoids to be assessed in that sample. The curve was prepared daily by diluting portions of the starting solutions to the appropriate proportions for the samples being analyzed.

The concentrations of stock standard solutions were determined spectrophotometrically on the basis of the published absorptivity value ($E^{1\%}_{1cm}$) in hexane, ethanol, or petroleum ether (De Ritter and Purcell, 1981). Values used and the wavelength maxima were as follows: β-carotene, 2592 at 453 nm; α-carotene, 2800 at 444 nm; γ-carotene, 3100 at 462 nm; lutein, 2550 at 445 nm; zeaxanthin, 2540 at 450 nm; β-cryptoxanthin, 2386 at 452 nm; lycopene, 3450 at 472 nm; and phytoene, 1250 at 285 nm. All standard solutions were stored in the dark at −25 °C under nitrogen, and their concentrations were recalibrated monthly.

RESULTS AND DISCUSSION

Tables I–III show the carotenoid composition of fresh Spanish vegetables, in both raw and cooked form in those that customarily undergo some culinary process prior to consumption. The tables specify both their common and their scientific names (Código Alimentario Español, 1985) for easier identification of the food item. The same starting batch was used for the analyses of both raw and cooked samples. Thus, variations in the carotenoid concentrations due to cultivar, growing season, location, and degree of ripeness could be ruled out (Speek et al., 1988; Mercadente and Rogríguez-Amaya, 1991; Khachik et al., 1991), and the effect of cooking on the carotenoids could be more accurately assessed.

The vegetables presented in Tables I–III are grouped according to the color of the edible portion: green, red-orange, and yellowish-white, in agreement with the associations used in epidemiologic studies (Ziegler et al., 1987; Le Marchand et al., 1989; Harris et al., 1991). Cucumbers and squash are included in the yellowish-white group since the green portion is removed from them for their consumption.

Lutein and/or zeaxanthin and β-carotene could be observed in all of the vegetables analyzed, while cantha-xanthin and β-apo-8′-carotenal were not detected in any of the samples.

Different epidemiological studies on cancer have assigned a protective role against the disease to the cruciferous vegetables (Graham et al., 1978; Haenszel et al., 1980), although this effect is probably due to other components such as indoles and phenols rather than to the carotenoids they contain (Le Marchand et al., 1989). The vegetables of the genus *Brassica* that we analyzed had low (Brussels sprouts) or very low (cabbage, red cabbage, and cauliflower) lutein and β-carotene contents.

In the green and yellowish-white groups, lutein predominated over β-carotene. The red-orange group had a more complicated carotenoid profile, with other carotenoids being more abundant than lutein (β-carotene in sweet red peppers, lycopene in tomatoes, α- and β-carotene in carrots).

The presence of zeaxanthin (confirmed using system II) was detected in spinach, sweet red peppers, cabbage, red cabbage, and potatoes, with traces in cauliflower and onions. In all of these samples, lutein and zeaxanthin were present jointly except in sweet red peppers, where only zeaxanthin was shown.

β-Cryptoxanthin was present in sweet red peppers and squash, with traces appearing in red cabbage and potatoes. α-Carotene was detected in green beans, carrots, and potatoes, while lycopene and γ-carotene were characteristic in the three varieties of tomato.

Table I. Carotenoid Content (Expressed in Micrograms per 100 g) of Edible Portion (Wet Weight) of Raw and Cooked Green Vegetables[a]

	moisture, %	non-provitamin A			provitamin A				RE,[b] μg
		lutein	zeaxanthin	lycopene	β-cryptoxanthin	γ-carotene	α-carotene	β-carotene	
lettuce (*Lactuca sativa*, L.)									
leaf type	95.0	340 ± 17						172 ± 8	29
iceberg type	96.1	140 ± 3						48 ± 2	8
artichokes (*Cynara scolymus*, L.)									
raw	83.7	163 ± 15						47 ± 5	8
cooked (30 min)	89.4	275 ± 23						59 ± 1	10
Brussel sprouts (*Brassica oleracea*, L.)									
raw	83.8	185 ± 19						77 ± 10[c]	13
cooked (25 min)	82.8	468 ± 36						162 ± 18[c]	27
green beans (*Phaseolus vulgaris*, Savi)									
raw	90.9	365 ± 7					35 ± 2	166 ± 10	28
cooked (35 min)	93.4	487 ± 5					79 ± 12	238 ± 15	40
asparagus (green) (*Asparragus officinalllis*, L.)									
raw	93.4	609 ± 20						320 ± 50	53
cooked (25 min)	92.2	738 ± 25						387 ± 49	65
beet (stalk + leaves) (*Beta vulgaris*, L.)									
raw	93.4	1503 ± 101						1095 ± 61	183
cooked (35 min)	95.1	1960 ± 85						1360 ± 34	227
green peppers (*Capsicum annuum*, L.)									
raw	92.6	341 ± 16						205 ± 11	34
cooked (25 min)	94.1	377 ± 83						255 ± 10	43
spinach (*Spinacia oleracea*, L.)									
raw	91.6	4229 ± 1310	377 ± 103					3254 ± 330[c]	542
cooked (10 min)	91.7	6422 ± 1190	564 ± 75					4626 ± 346[c]	771

[a] HPLC system I: lycopene, β-cryptoxanthin, γ-carotene, α-carotene, β-carotene. HPLC system II: lutein, zeaxanthin, canthaxanthin, β-apo-8′-carotenal. [b] RE, (μg of β-carotene/6) + (μg of other provitamin carotenoids/12). [c] *all-trans*-β-carotene.

Table II. Carotenoid Content (Expressed in Micrograms per 100 g) of Edible Portion (Wet Weight) of Raw and Cooked Red-Orange Vegetables[a]

	moisture, %	non-provitamin A			provitamin A				RE,[b] (μg)
		lutein	zeaxanthin	lycopene	β-cryptoxanthin	γ-carotene	α-carotene	β-carotene	
tomato (*Solanum lycopersicum*, Mill.)									
common type	93.6	52 ± 12		2116 ± 583		143 ± 35		494 ± 124	94
Canary Islands type	93.6	44 ± 1		1604 ± 283		37 ± 4		443 ± 37	77
pear type	93.6	72 ± 7		62273 ± 7944		161 ± 22		393 ± 39	79
red peppers (*Capsicum annuum*, L.)									
raw	91.4		148 ± 38		199 ± 17			414 ± 33	86
cooked (38 min)	90.3		197 ± 27		243 ± 11			693 ± 6	136
carrots (*Daucus carota*, L.)									
raw	88.1	288 ± 33					2895 ± 276	6628 ± 45[c]	1346
cooked (33 min)	89.7	273 ± 35					3245 ± 128	8162 ± 364[c]	1631
red cabbage (*Brassica oleracea*, L.)									
raw	88.2	8 ± 2	tr[d]		tr			3 ± 0.2[c]	0.6
cooked (38 min)	91.3	23 ± 1	4 ± 1		tr			7 ± 1[c]	1.2
red peppers saponified extracts									
raw			289 ± 37		251 ± 24			478 ± 50	101
cooked			390 ± 90		371 ± 36			768 ± 93	159

[a] HPLC system I: lycopene, β-cryptoxanthin, γ-carotene, α-carotene, β-carotene. HPLC system II: lutein, zeaxanthin, canthaxanthin, β-apo-8′-carotenal. [b] RE, (μg of β-carotene/6) + (μg of other provitamin carotenoids/12). [c] *all-trans*-β-carotene. [d] tr, trace.

In Tables I–III the values are given for retinol equivalents, calculated on the basis of the carotenoid concentrations quantified in the samples and following the guidelines of the Food and Nutrition Board (1980). In this study, it was generally observed that the provitamin A activity in the green and yellowish-white vegetables (with the exception of green beans) is due almost exclusively to β-carotene, while in the red-orange vegetable group the provitamin A activity is due not only to β-carotene but to other carotenoids with provitamin A activity (β-cryptoxanthin, α-carotene, and γ-carotene).

The values for retinol equivalents presented here are, in general, lower than those reported in the current Food Composition Tables (Paul and Southgate, 1978; Souci et al., 1989; Instituto de Nutrición, 1980) except in carrots, where it is similar, probably due to the fact that the most

Table III. Carotenoid Content (Expressed in Micrograms per 100 g) of Edible Portion (Wet Weight) of Raw and Cooked Yellowish-White Vegetables[a]

	moisture, %	non-provitamin A			provitamin A				RE,[b] μg
		lutein	zeaxanthin	lycopene	β-cryptoxanthin	γ-carotene	α-carotene	β-carotene	
cucumber (*Cucumis sativus*, L.)	95.7	16 ± 1						11 ± 1	2
squash (*Cucurbita pepo*, L. var. Medellusa, Alef.)									
raw	94.6	100 ± 25			tr[c]			23 ● 2	4
cooked (15 min)	94.5	118 ± 13			4			26 ± 2	5
potato (Early) (*Solanum tuberosum*, L.)									
raw	80.3	12 ● 1	4 ± 0.5		tr		tr	1 ± 0.2	0.2
cooked (20 min)	81.9	44 ± 1	21 ± 0.5		tr		tr	1.5 ± 0.3	0.3
onion (*Allium cepa*, L.)									
raw	90.9	2 ± 0.5	tr					1 ● 0.4	0.2
cooked (38 min)	93.2	5 ± 0.5	tr					3 ± 0.3	0.5
cabbage (*Brassica oleracea*, L.)									
raw	89.0	59 ± 2	6 ± 2					22 ± 2[d]	4
cooked (25 min)	90.8	93 ± 20	6 ± 3					33 ● 3[d]	5
cauliflower (*Brassica oleracea*, L.)									
raw	91.1	4 ± 0.4						2 ± 0.2[d]	0.4
cooked (30 min)	92.5	15 ± 1	tr					7 ± 1[d]	1.2
squash saponified extracts									
raw		108 ± 13			6 ± 2			21 ± 2	4.1
cooked		169 ± 10			11 ± 1			28 ● 2	5.6

[a] HPLC system I: lycopene, β-cryptoxanthin, γ-carotene, α-carotene, β-carotene. HPLC system II: lutein, zeaxanthin, canthaxanthin, β-apo-8′-carotenal. [b] RE, μg of β-carotene/6) + (μg of other provitamin carotenoids/12). [c] tr, trace. [d] *all-trans*-β-carotene.

abundant carotenoids in this vegetable (β- and α-carotene) are of provitamin nature.

The comparison of our findings with those of other authors that have used HPLC in their analyses (Bushway and Wilson, 1982; Bureau and Bushway, 1986; Khachik et al., 1986; Khachik and Beecher, 1987; Varo et al., 1989; Heinonen et al., 1989; Tee and Lim, 1991) also reveals disparity, probably due to factors such as the variety being studied, season, and degree of ripeness. With respect to variability, like other authors (Heinonen et al., 1989), we have observed variations (data not shown) in the concentration of carotenoids detected in a given vegetable (onions, tomtoes, carrots) depending on the season of the year and even within the same season of consecutive years (carrots, tomatoes). The variety-dependent variation in the carotenoid content can be clearly observed in the respective comparisons of the two varieties of lettuce (Table I) and the three varieties of tomato (Table II).

In addition to the carotenoids shown in Tables I–III phytoene and phytofluene were detected in carrots, sweet red peppers, and the three varieties of tomato. Phytoene was identified by retention time, by injection with standard, and by stop-flow scan at the top of the peak, while phytofluene was tentatively identified by stop-flow scan (Granado et al., 1991). The phytoene concentration (expressed as wet substance) in carrot samples (saponified) was 1769 (raw) and 1197 μg % (cooked), in red peppers (saponified) 721 (raw) and 1034 μg % (cooked), in common tomatoes 923 μg %, in pear tomatoes, 2795 μg %, and in Canary Island tomatoes 489 μg %.

Effect of Cooking. The majority of the vegetables were analyzed after undergoing a process of boiling since they are customarily consumed following some culinary process. The data are shown in Tables I–III together with the results of the assessment of uncooked samples.

In our study, while boiling did not alter the carotenoid profile of the samples, it did result in an increase in the amounts of carotenoids quantified as compared to that of the raw state. This increase occurred regardless of the type of carotenoid, the vegetable, and whether or not the sample had been saponified. By contrast, other authors have described losses in carotenoid contents when the samples were subjected to different culinary processes (Speek et al., 1988; Khachik et al., 1991). Although cooking procedures may result in loss of carotenoids in some vegetables, heat treatment increases the chemical extractability of carotenoids (Bushway and Wilson, 1982; Khachik and Beecher, 1987; Varo et al., 1989).

When the carotenoid concentration is expressed in percent retention (Varo et al., 1989), we obtain increases ranging between 100 and 600% in terms of dry substance. Cooking produced increases in carotenoid values ranging as follows: lutein, from 103 (asparagus) to 409% (cauliflower); zeaxanthin, from 116 (cabbage) to 618% (potatoes); β-cryptoxanthin, from 107 (red peppers) to 326% (potatoes); α-carotene, from 129 (carrots) to 313% (green beans); and β-carotene, from 101 (asparagus) to 344% (onions). The increment varies according to the sample and carotenoid being studied, being greater in those vegetables in which the carotenoid concentration is lower (e.g., potatoes, cauliflower, red cabbage, or artichokes).

To assess the possible losses in carotenoid concentration due to handling of the sample, but not related to efficiency of the extraction method, echinenone or retinyl palmitate was added to half of the analyzed subsamples at the beginning of the extraction process as internal standard. In our analyses, echinenone eluted at the midpoint of the chromatogram, between the xanthophylls and the carotenes, and was useful when there were no interferences from the vegetable extract in that zone of the chromatogram and, for this reason, was not useful with the nonsaponified green vegetable extracts. Retinyl palmitate may be a good internal standard for β-carotene in nonsaponified extracts, since it elutes after β-carotene and does not interfere with its cis isomers. To correct the concentrations, the heights of the internal standard peaks in the samples were compared with the peaks of the internal standards obtained after injection of the standard solution. On the other hand, recovery studies were not carried out since the isolation of carotenes from vegetable tissue is

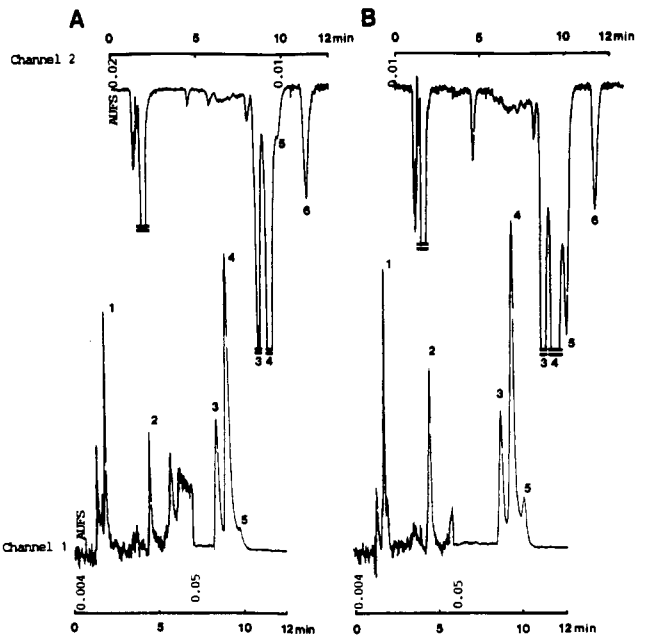

Figure 2. Chromatogram of carrot extract: (A) raw; (B) cooked. A Spheri-5-ODS column was used, mobile phase (system I). Detection was at 450 nm (channel 1), maxplot 340–370–286–450 nm (channel 2). Peak identification: 1, lutein; 2, echinenone; 3, α-carotene; 4, *all-trans*-β-carotene; 5, 13-*cis*-β-carotene; 6, phytoene.

not analytically comparable with the separation of added free β-carotene (Expert Group on Vitamin Determination, 1986).

The saponification step was carried out in most of the samples analyzed to evaluate the presence of carotenoid esters and remove any substances which could interfere with the chromatogram. In most cases, this process resulted in a loss of carotenoids, which occurred in greater proportion in the xanthophylls than in the carotenes, as reported by other authors (Khachik et al., 1986). This loss in the concentration was partially corrected when the sample was quantified on the basis of a calibration curve, which was also subjected to a process of saponification. Nevertheless, in the case of squash and sweet red pepper, saponification permitted an increase in the concentration of the carotenoids quantified owing to the presence of carotenoid esters in the sample. In general, saponification may be an interesting step in the quantification of carotenoids in vegetables as it eliminates substances that interfere in their determination (e.g., chlorophylls, pheophytins). Since the process does not affect all of the carotenoids homogeneously and has not been carried out in all of the samples, the data concerning the saponificated extracts are not presented in the tables.

Several vegetables (Brussels sprouts, spinach, carrots, red cabbage, cabbage, and cauliflower) were analyzed using a Spheri-5-ODS column capable of separating *all-trans*-β-carotene and *cis*-β-carotenes (Granado et al., 1991). In all of these samples, peaks eluting after β-carotene were detected and were tentatively identified as *cis*-β-carotenes. Thus, the concentration of β-carotene in these samples corresponds to *all-trans*-β-carotene and not to total β-carotene. Owing to the low concentration of these isomers in the samples, they were not quantified (except in carrots). However, 13-*cis*-β-carotene was tentatively identified in carrots and 13-*cis*- and 9-*cis*-β-carotene in spinach.

These cis peaks were detected both in raw and in cooked extracts (Figure 2), possibly as a consequence of the extraction process or perhaps they were present in natural form in the sample (Quackenbush, 1987). The quantification of cis isomers was only done in carrots (although not taken into account for retinol equivalents), revealing them to be twice as abundant in the cooked sample as in the raw extract.

A great number of factors (genetic, environmental, and culinary) affect the determination of carotenoids in foods. For these data on carotenoid contents in vegetables to be included in the Food Composition Tables, it would be necessary to carry out a greater number of analyses for each vegetable, from different geographical sources and at different times of year. Our results are not even necessarily representative of the carotenoid concentrations in the vegetables consumed in the rest of our country.

With respect to the provitamin A activity of these vegetables, the cooking process, which produces an increase in the carotenoids quantified, also raises the values for the retinol equivalents calculated for each vegetable, a fact which should be taken into account in the preparation of Food Composition Tables and diets.

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LITERATURE CITED

Bureau, J. L.; Bushway, R. J. HPLC determination of carotenoids in fruits and vegetables in the United States. *J. Food Sci.* **1986**, *51*, 128–130.

Bushway, R. J.; Wilson, A. M. Determination of α- and β-carotene in fruit and vegetables by high performance liquid chromatography. *Can. Inst. Food Sci. Technol. J.* **1982**, *15*, 165–169.

Código Alimentario Español. Boletín Oficial del Estado: Madrid, Spain, 1985.

Comstock, G. W.; Helzlsouer, K. J.; Bush, T. L. Prediagnostic serum levels of carotenoids and vitamin E as related to subsequent cancer in Washington County, Maryland. *Am. J. Clin. Nutr.* **1991**, *53*, 260S–264S.

De Ritter, E.; Purcell, A. E. Carotenoid analytical methods. In *Carotenoids as colorants and vitamin A precursors*; Bauernfeind, J. C., Ed.; Academic Press: London, 1981; Chapter 10.

Expert Group on Vitamin Determination. COST 91. In *Methods for the determination of vitamins in food. Recommended by COST 91*; Brubacher, G., Müller-Mulot, W., Southgate, D. A. T., Eds.; Elsevier: England, 1986; Chapter 3.

Food and Nutrition Board. *Recommended dietary allowances*; National Academy of Science: Washington, DC, 1980; Chapter 7.

Graham, S.; Dayal, H.; Swanson, M.; Mittelman, A.; Wilkinson, G. Diet in the epidemiology of cancer of the colon and rectum. *J. Natl. Cancer Inst.* **1978**, *61*, 709–714.

Granado, F.; Olmedilla, B.; Blanco, I.; Rojas-Hidalgo, E. An improved HPLC method for the separation of fourteen carotenoids, including 15-/13- and 9-cis-β-carotene isomers, phytoene and phytofluene. *J. Liq. Chromatog.* **1991**, *14*, 2457–2475.

Haenszel, W.; Locke, F. B.; Segi, M. A case-control study of large bowel cancer in Japan. *J. Natl. Cancer Inst.* **1980**, *64*, 17–22.

Harris, R. W. C.; Key, T. J. A.; Silcocks, D. B.; Wald, N. J. A case-control study of dietary carotene in men with lung cancer and in men with other epithelial cancers. *Nutr. Cancer* **1991**, *15*, 63–68.

Heinonen, M. I.; Ollilainen, V.; Linkola, E. K.; Varo, P. T.; Koivistoinen, P. E. Carotenoids in Finnish foods: vegetables, fruits and berries. *J. Agric. Food Chem.* **1989**, *37*, 655–659.

Instituto de Nutrición. *Tablas de Composición de Alimentos*; Consejo Superior de Investigaciones Científicas (CSIC): Madrid, Spain, 1980.

Katrangi, N.; Kaplan, L. A.; Stein, E. A. Separation and quantitation of serum β-carotene and other carotenoids by

high performance liquid chromatography. *J. Lipid Res.* **1984**, *25*, (4), 400–406.

Khachik, F.; Beecher, G. R. Application of a C-45-β-carotene as an internal standard for the quantification of carotenoids in yellow/orange vegetables by liquid chromatography. *J. Agric. Food Chem.* **1987**, *35*, 732–738.

Khachik, F.; Beecher, G. R.; Whittaker, N. F. Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J. Agric. Food Chem.* **1986**, *34*, 603–616.

Khachik, F.; Beecher, G. R.; Goli, M. B.; Lusby, W. R. Separation, identification and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromatography. *Pure Appl. Chem.* **1991**, *63*, 71–80.

LeMarchand, L.; Yoshizawa, C. N.; Kolonel, L. N.; Hankin, J. H.; Goodman, M. T. Vegetable consumption and lung cancer risk: A population-based case-control study in Hawaii. *J. Natl. Cancer Inst.* **1989**, *81*, 1158–1164.

Malo, M.; del Valle, M. *Guía práctica del consumo de alimentos*; Gobierno Regional de Cantabria, Ministerio de Sanidad y Consumo: Santander, Spain, 1987; Chapter 4.

Mercadante, A. Z.; Rodríguez-Amaya, D. B. Carotenoid composition of a leafy vegetable in relation to some agricultural variables. *J. Agric. Food Chem.* **1991**, *39*, 1094–1097.

Ministerio de Agricultura, Pesca y Alimentación. *Consumo alimentario en España 1987*; Secretaría General Técnica: Madrid, Spain, 1988; Chapter 3.

Moreiras, O.; Carbajal, A.; Perea del Pino, I. M. *Evolución de los hábitos alimentarios en España*; Ministerio de Salud y Consumo, Secretaria General Técnica: Madrid, Spain, 1990.

Olmedilla, B.; Granado, F.; Rojas-Hidalgo, E.; Blanco, I. A rapid separation of ten carotenoids, three retinoids, alpha-tocopherol and d-α-tocopherol acetate by HPLC and its application to serum and vegetable samples. *J. Liq. Chromatogr.* **1990**, *13*, 1455–1483.

Paul, A. A.; Southgate, D. A. T. *The composition of foods*; HMSO: London, 1978.

Quackenbush, F. W. Reverse phase HPLC separation of cis- and trans-carotenoids and its application to β-carotenes in food materials. *J. Liq. Chromatogr.* **1987**, *10*, 643–653.

Souci, S. W.; Fachmann, W.; Kraut, H. *Food composition and nutrition tables 1989/1990*; Wissenschaftliche Verlagsgesellschaft: Stuttgart, 1989.

Speek, A. J.; Speek-Saichua, A.; Schreurs, W. H. P. Total carotenoid and β-carotene contents of Thai vegetables. *Food Chem.* **1988**, *27*, 245–257.

Tee, E.-S.; Lim, C.-L. Carotenoid composition and content of Malaysian vegetables and fruits by the AOAC and HPLC methods. *Food Chem.* **1991**, *41*, 309–339.

Varo, P.; Mattila, M.; Piironen, V.; Nironen, P.; Koivistoinen, P. Effects on food preparation of natural antioxidant levels in foods. In *Advances in food technology. Proceedings of the II World Congress of Food Technology*, Barcelona, 1987; Primo Yúfera, E., Fito Manpoey, P., Eds.; II World Congress of Food Technology: Barcelona, 1989; Vol. 2, Chapter 1.

Ziegler, R. G. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. *J. Nutr.* **1989**, *119*, 116–122.

Ziegler, R. G.; Wilcox, H. B. III; Mason, T. J.; Bill, J. S.; Virgo, P. W. Seasonal variation in intake of carotenoids and vegetables and fruits among white men in New Jersey. *Am. J. Clin. Nutr.* **1987**, *45*, 107–114.

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Registry No. Lutein, 127-40-2; zeaxanthin, 144-68-3; lycopene, 502-65-8; β-cryptoxanthin, 472-70-8; γ-carotene, 472-93-5; α-carotene, 7488-99-5; β-carotene, 7235-40-7.