Comparison of Two Methods for Determining the Vitamin A Value of Clingstone Peaches

Susan E. Gebhardt,* E. R. Elkins, and Johanna Humphrey

The vitamin A activity of fruits and vegetables is due to the amount of the various provitamin A carotenoids that are present. Recent studies, however, have shown that reported values for vitamin A content in some red and yellow fruits and vegetables may be erroneously high due to the inclusion of carotenoids without activity in the vitamin A calculations. The work reported in this paper relates to clingstone peaches and compares data obtained by use of a modified AOAC carotene method (method I) with a method that separates and quantitates all provitamin A carotenoids individually (method II, individual isomer method). Further, method II eliminates those pigments without activity from the calculations. Vitamin A content of peaches is indicated to be almost twice as high by method I as by method II. The results also suggest that much work needs to be done to determine more accurately the vitamin A values of yellow fruits and vegetables.

The vitamin A activity of fruits and vegetables is due to the amount of the various provitamin A carotenoids that are present. Analytical determination of vitamin A activity has been based on the assumption that most of the provitamin A present is β-carotene. Some fruits and vegetables have considerable amounts of active carotenoids other than β-carotene, and these other carotenoids have approximately half the biological activity of β-carotene. To determine an accurate vitamin A value, the various active carotenoids must be separated from each other, identified, measured quantitatively, and weighted by their different biological activities (Roels, 1967). Any method which does not do this, but measures all provitamin A carotenoids as β-carotene, will overestimate vitamin A activity.

As part of a project designed to compare nutrients in canned fruits and vegetables of today with those of the 1940's, a comparison of two methods for determining the vitamin A value of clingstone peaches was undertaken. Peaches were used because they are known to have large amounts of provitamin A carotenoids other than β-carotene (Curl, 1959) and would be representative of many other red and yellow fruits and vegetables for which this is true.

The methods used were (I) a modified Association of Official Analytical Chemists method (1970)—although designed for fresh green plants and their silages, AOAC is at present the most widely used method for determining the vitamin A content of fruits and vegetables—and (II) a method which measures all provitamin A carotenoids individually, called the individual isomer method. These will be referred to as method I and method II, respectively.

EXPERIMENTAL SECTION

Sampling. Three commercial packs of clingstone peaches were investigated. Pack no. 1 was Halford variety, picked August 1972; pack no. 2 was Dixon variety, picked July 1973; and pack no. 3 was Carolyn variety, picked August 1973. All packs were grown and packed in California.

The peaches were washed, torque pitted, and lye peeled. Raw samples were taken for analyses before peach halves or slices were filled into cans, run through syrupers, and steam closed without exhausting. Half of the cans were run through a continuous cooker for 8.7–9.5 min at 226–230 °F and then the cans were water cooled about 5 min. The other half of the cans were processed in a still retort for 30 min at 212 °F and then water cooled to 90–100 °F. Two to three pounds of raw peaches or six cans were composited for analyses. Cans from each of the three packs and two processing techniques were analyzed immediately after processing and after 6, 12, and 18 months storage at room temperature.

Modified AOAC Method. Method I. The extraction procedure was the same for both methods, although a larger sample size, 100–200 g of blended peaches, was required for method II because a larger quantity of pigment is necessary for separation of isomers. For method I, a 25-g sample of blended peaches was used. Water was drawn off under suction, and the resulting thick paste was extracted with acetone until the extract was colorless. The acetone solution was transferred to a separatory funnel where the pigment was reextracted into petroleum ether and the acetone layer was discarded. The petroleum ether extract was washed with water, filtered through Na_2SO_4, and concentrated to about 10 mL by a rotary evaporator at room temperature.

For the separation of pigments by method I, a 10-cm column was packed under vacuum with an adsorbent of one part Mallinckrodt MgO to one part Hyflo Super Cel with 1 cm of Na_2SO_4 at the top of the column. It was found that a 1 to 3 ratio of MgO to Hyflo Super Cel could be used to speed the elution of the carotenoids through the column if care was taken to elute only those bands that were eluted from the 1:1 MgO–Hyflo Super Cel adsorbent. The column was prewetted with 25 mL of petroleum ether and the concentrated extract put on the column. As shown in Figure 1, pigments were washed through the column with 10% acetone in petroleum ether, which elutes carotenes and monohydroxy carotenoids that were collected and read at their peak absorptivity on a recording spectrophotometer. Polyhydroxy carotenoids (xanthophylls) were assumed to have remained on the column.

Individual Isomer Method. Method II. In order to measure the individual provitamin A carotenoids of peaches, the method of Sweeney and Marsh (1970) was modified. The original method only separates α- and β-carotene and their cis isomers. Since cryptoxanthin, a monohydroxy carotene, is one of the major provitamin A carotenoids in peaches, the method was modified to separate this pigment.

For the first separation of pigments in method II (Figure 2), a 10-cm column was packed with one part MgO and three parts Hyflo Super Cel. The carotenoid extract was

National Canners Association, Washington, D.C. 20036 (E.R.E., J.H.) and Consumer and Food Economics Institute, Agricultural Research Service, U.S. Department of Agriculture, Hyattsville, Maryland 20782 (S.E.G.).

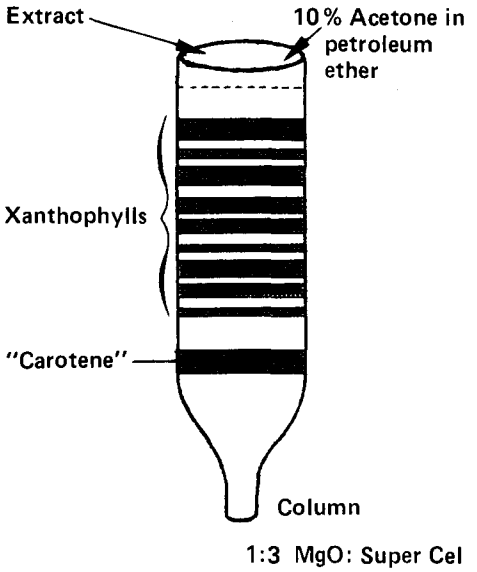

Figure 1. Separation of carotenoids by method I.

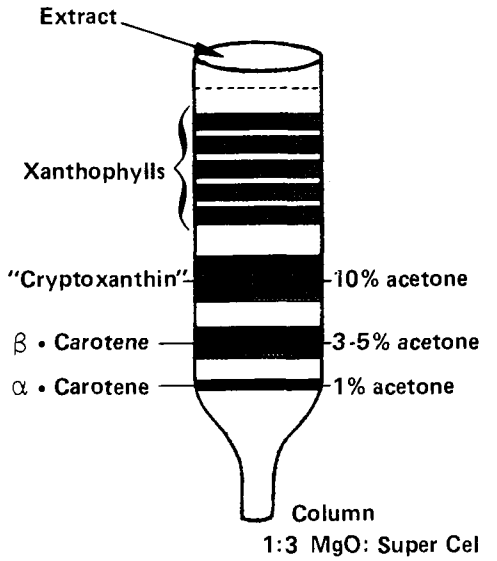

Figure 2. Initial separation of carotenoids by method II.

placed on the column, and pigments were eluted with gradually increasing concentrations of acetone in petroleum ether. α-Carotene was eluted first with 1% acetone in petroleum ether; then β-carotene, with 3–5% acetone in petroleum ether; and cryptoxanthin, with 10% acetone in petroleum ether. It was found necessary to transfer the carotenoid fraction eluted by the 10% acetone to another column packed with one part $Mg(OH)_2$ and six parts $Ca(OH)_2$ and to elute it with 1–3% acetone in petroleum ether (Figure 3). This fraction separated into many bands. The neocryptoxanthin and *trans*-cryptoxanthin bands were eluted together and read on a recording spectrophotometer. The polyhydroxy pigments were retained on the column.

There is only a trace of α-carotene in peaches, so there was no need to try to separate isomers of α-carotene. The β-carotene fraction eluted from the first column was transferred to a second column packed with 1:6 mixture of $Mg(OH)_2$ and $Ca(OH)_2$. The isomers were separated by washing the column with 1.5% *p*-methylanisole in petroleum ether. The β-carotene band separated into the cis isomers, neo-β-carotenes B, D, and U, and *all-trans*-β-carotene.

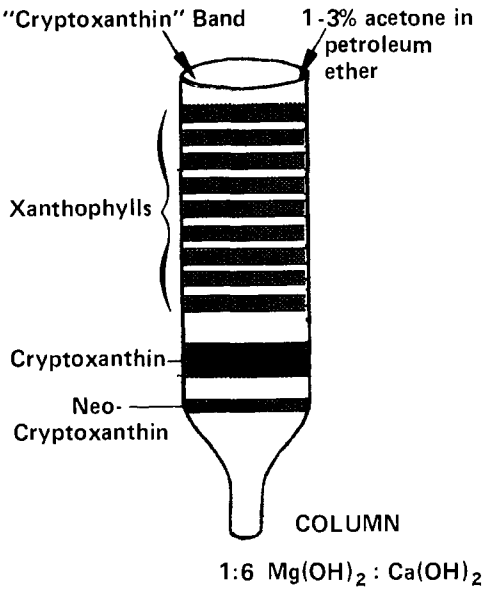

Figure 3. Extra chromatographic separation of the "cryptoxanthin" band eluted during the initial separation of carotenoids for method II.

Table I. Absorbance Values of Provitamin A Carotenoids

Isomer	Absorbance: Maximum (nm)	Absorbance: $E^{1\%}_{1cm}$
all-trans-α-Carotene	447	2640
all-trans-β-Carotene	450	2480
Neo-β-carotene B	444	1930
Neo-β-carotene D	441	
Neo-β-carotene U	449	2360
Cryptoxanthin[a]	451	2160

[a] Personal communication with J. P. Sweeney.

Table II. Provitamin A Carotenoid Content of Peaches as Determined by Method II without and with Extra Chromatographic Step

Provitamin A carotenoids	Without: μg/100 g	Without: % of total	With: μg/100 g	With: % of total
β-Carotene and isomers	310	18	310	63
"Cryptoxanthin"	1433	82	184	37
Total	1743		494	

In Table I are listed the absorbance values and specific absorption coefficients for the carotenoids that were studied (Sweeney and Marsh, 1970).

RESULTS AND DISCUSSION

In the preliminary procedures, when method II was run without the separation of the "cryptoxanthin band" eluted from the MgO–Hyflo Super Cel column with 10% acetone, the cryptoxanthin value was extremely high. When the purity of this band was checked by eluting it from the $Mg(OH)_2$–$Ca(OH)_2$ column with 1–3% acetone in petroleum ether, it separated into many bands, indicating that polyhydroxy carotenoids had also been eluted by the 10% acetone. Results without the extra separation of the "cryptoxanthin band" and with the extra separation of a test sample are given in Table II. It is evident that the 10% acetone in petroleum ether, which is used for method I, elutes carotenoids other than cryptoxanthin. These polyhydroxy carotenoids do not have vitamin A activity.

Table III. Calculating Effective Carotene for Raw Peaches from Results of Method II

Isomer	Biological activity, %	Amount, μg/100 g	Effective carotene, μg/100 g
all-trans-β-Carotene	100	186	186
Neo-β-carotene B	53	51	27
Neo-β-carotene U	38	18	7
Cryptoxanthin	50	201	101
		Total	321

Table IV. Amounts of Total Carotene in Raw and Canned Peaches as Analyzed by Methods I and II and Effective Carotene Calculated from the Results of Method II

Method	Raw,[a] μg/100 g	Canned,[b] μg/100 g	Retention, %
Method I	1790	552	31
Method II	456	386	85
Effective carotene	321	270	84

[a] Mean of three samples. [b] Mean of 24 samples.

Therefore, the extra chromatographic separation of cryptoxanthin from inactive carotenoids also eluted by the 10% acetone was included as part of method II.

Analyses of the canned samples were done on the total can contents, but for the comparison of raw and canned peaches the values were corrected to exclude the added syrup—values are reported for 100 g of canned peaches without syrup.

Before comparing results of methods I and II, an effective carotene value was calculated from results of method II by multiplying the amount of each isomer by its percentage of biological activity in relation to β-carotene (Table III). *all-trans*-β-Carotene is considered to be 100% active. Neo-β-carotene B has 53%, neo-β-carotene U has 38%, and cryptoxanthin and its isomer have about 50% of the activity of *all-trans*-β-carotene (Zechmeister, 1962). Amounts of effective carotene for each isomer were then added together for a value that is equivalent to micrograms of *all-trans*-β-carotene.

Average carotene values for methods I and II and calculated effective carotene for raw and canned peaches are reported in Table IV. Amount of total carotene as determined by method II is lower than method I for both raw and canned peaches. Calculated effective carotene values are even lower because isomers other than *trans*-β-carotene which were measured have been corrected for their lesser activity in relation to *trans*-β-carotene. The retention of carotene in canned peaches is only 31% using method I, but the retention is 85% when method II is used. This would seem to indicate that the greatest changes during canning occurred in the polyhydroxy carotenoids which were measured by method I but were not measured by method II.

It has been reported that, during canning, the proportion of *all-trans*-β-carotene decreases with a subsequent rise in the less active cis isomers (Sweeney and Marsh, 1971; Lee and Ammerman, 1974). We did not find this to be true for peaches. In Table V micrograms and percentage amounts for each isomer are given. α-Carotene and neo-β-carotene D are not shown since they were present in only trace amounts. There is a decrease in total amount of trans isomers from the raw to canned peaches, but there is not a marked change in the percentage composition of the isomers. Panalaks and Murray (1970) investigated the α- and β-carotene isomers of five vegetables and peaches. They found that canning did result in changes in the isomeric composition of all products except green beans and peaches.

Table V. Carotenoid Isomers of Raw and Canned Peaches

Isomers	Raw,[a] μg/100 g	%	Canned,[b] μg/100 g	%
Neo-β-carotene B	51	11	36	9
all-trans-β-Carotene	186	41	157	41
Neo-β-carotene B	18	4	24	6
Cryptoxanthin	201	44	169	44
Total	456		386	

[a] Mean of three samples. [b] Mean of 24 samples.

Table VI. Vitamin A Value of Clingstone Peaches

	Method I[a]		Method II[a]	
	IU	% U.S. RDA	IU	% U.S. RDA
Raw	2983	60	535	11
Canned	579	12	282	6

[a] Per 100 g of total can contents.

Nutritional Implications. The vitamin A value of foods is usually reported in International Units (IU). To calculate IU from micrograms of carotenoids, by definition 0.6 μg of *all-trans*-β-carotene or 1.2 μg of other provitamin A carotenoids are equal to 1 IU (Food and Nutrition Board, 1974). It would be more accurate to use the specific biological activity of each isomer as was done for calculating effective carotene since some have over half and others less than half of the activity of β-carotene, but using the 1.2 equivalent is the accepted procedure.

Many of the vitamin A analyses of foods that are being done today are for nutrition labeling purposes. Values are reported as percent of the U.S. Recommended Daily Allowance (U.S. RDA), which is 5000 IU for vitamin A (Federal Register, 1973).

In Table VI, IU values and percent U.S. RDA are reported for 100 g of raw and canned peaches analyzed by the two methods. Percent of the U.S. RDA is much higher for raw peaches when method I is used than when method II is used: 60% vs. 11%. The difference between the two methods is not as great for canned peaches: 12% for method I and 6% for method II, but it is still substantial.

From these results it is evident that when analyzing fruits and vegetables with large amounts of carotenoids other than β-carotene, the method of analysis will significantly affect the nutritional labeling claim.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical advice of J. P. Sweeney on the modification of the isomer method.

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 11th ed, Washington, D.C., 1970.
Curl, A. L., *Food Res.* **24**, 413 (1959).
Federal Register 38(49), 6960 (March 14, 1973).
Food and Nutrition Board, National Research Council, National Academy of Sciences, "Recommended Dietary Allowances", 8th ed, Washington, D.C., 1974.
Lee, W. G., Ammerman, G. R., *J. Food Sci.* **39**, 1188 (1974).
Panalaks, T., Murray, T. K., *Can. Inst. Food Sci. Technol. J.* **3**, 145 (1970).
Roels, O. A., "The Vitamins", Sebrell, W. H., Harris, R. S., Ed., Academic Press, New York, N.Y., 1967, p 114.
Sweeney, J. P., Marsh, A. C., *J. Assoc. Off. Anal. Chem.* **53**, 937 (1970).
Sweeney, J. P., Marsh, A. C., *J. Am. Diet. Assoc.* **59**, 238 (1971).

Zechmeister, L. "Cis-Trans Isomeric Carotenoids, Vitamins A and Arylpolyenes", Academic Press, New York, N.Y., 1962.

Received for review November 17, 1976. Accepted January 21, 1977. Work conducted under contract No. 12-14-100-11054(62) awarded to the National Canners Association Research Foundation by the Consumer and Food Economics Institute, Agricultural Research Service, U.S. Department of Agriculture. Presented before the First Chemical Congress of the North American Continent, Mexico City, Mexico, Dec 1975.

Reactions of Phosphoproteins in Alkaline Solutions

Lourminia C. Sen, Elvira Gonzalez-Flores, Robert E. Feeney, and John R. Whitaker*

Phosvitin, an egg yolk protein of mol wt 35 500 with 10% phosphate in the form of O-phosphoserine residues (reported value of 119 phosphoserine and one phosphothreonine residues), was used in model systems to investigate the effects of various experimental conditions on the rates of β elimination of phosphate from the phosphoserine residues and addition of indogenous nucleophiles to the double bond of the dehydroalanines formed. At low concentrations of phosvitin ($1–10 \times 10^{-6}$ M) the rates were independent of phosvitin concentration but directly dependent on the hydroxide ion concentration of the solution. The activation energies of the β-elimination and addition reactions were 20.2 and 25.0 kcal/mol, respectively. The rate of the β-elimination reaction was increased at increased ionic strengths and was markedly increased in the presence of $CaCl_2$. At 1.12×10^{-3} M $CaCl_2$, the rate of β elimination was 20 times faster than in the absence of $CaCl_2$. The rate of the addition reaction was not significantly affected by $CaCl_2$ or ionic strength.

Sodium hydroxide, on the GRAS (Generally Recognized as Safe) list of chemicals, is widely used in the food processing industry in the neutralizing, peeling, solubilizing, detoxifying (Screenivasamurthy, 1967), and texturizing (Gutcho, 1973) of foods. However, it is known that a number of reactions involving proteins occur in alkaline solution.

Alkali treatment of proteins has been shown to cause losses in serine, threonine, cystine, arginine, lysine, and an increase in ornithine (Pickering and Li, 1964; Geschwind and Li, 1964; Zeigler et al., 1967; Mellet, 1968; Blackburn, 1968; DeGroot and Slump, 1969; Parisot and Derminot, 1970; Whiting, 1971; Gottschalk, 1972; Provansal et al., 1975). Amino acid residues in proteins undergo racemization in alkaline solution more readily than do the free amino acids. These racemization reactions have been known for a long time (Dakin, 1912; Levene and Bass, 1928) and occur at different rates depending on the particular amino acid residue and experimental conditions (Neuberger, 1948; Hill and Leach, 1964; Pickering and Li, 1964; Pollock and Frommhagen, 1968; Tannenbaum et al., 1970).

New amino acids are formed during alkali treatment of proteins. Ornithine arises from the hydrolysis of the guanido group of arginine (Ziegler et al., 1967). Lysinoalanine residues have been reported in several proteins following alkali treatment (Patchornik and Sokolovsky, 1964; Bohak, 1964; Ziegler, 1964; Corfield et al., 1967; Robson and Zaidi, 1967; Whiting, 1971). Lanthionine has been identified in alkali-treated wool (Corfield et al., 1967; Asquith and García-Domínquez, 1968) as well as in other sulfur-containing proteins (Nashef et al., 1977). β-Aminoalanine has also been identified in an hydrolyzate of alkali-treated wool (Asquith et al., 1969) as has ornithinoalanine in alkali-treated proteins (Ziegler et al., 1967).

The new amino acids, lysinoalanine, lanthionine, ornithinoalanine, and β-aminoalanine, formed in proteins by alkali treatment can be explained by the initial formation of the common intermediate dehydroalanine (2-aminopropenic acid) from the decomposition of cystine, *O*-phosphoserine, and *O*-glycosylserine residues. The addition of the ϵ-amino group of a lysine residue, the δ-amino group of an ornithine residue, the sulfhydryl group of a cysteine residue, or ammonia to the double bond of the dehydroalanine forms the new amino acids. Direct evidence for dehydroalanine formation during alkali treatment of proteins is available (Asquith and Carthew, 1972; Miro and García-Domínquez, 1973). While there is evidence for the destruction and formation of new amino acids during the alkali treatment of proteins, there is very little information on the effect of various experimental parameters on the rates of destruction of cystine and O-substituted serine and threonine residues and the formation of new products.

Our laboratory has undertaken a systematic study of the reactions of proteins in alkaline solutions. Initially, we have chosen to study the behavior of model proteins and peptides and then to extend these data to a study of more complex food systems. We have reported on the behavior of cystine residues (Nashef et al., 1977) and *O*-glycosylthreonine residues (Lee et al., 1977) in proteins in alkaline solution.

In the present paper, we examine the effect of alkaline solution on the phosphoprotein, phosvitin. This protein is ideally suited for these studies since it contains a reported 119 phosphoserine residues, only one phosphothreonine residue, no cystine or cysteine, and the carbohydrate moiety is alkali stable as it is attached to the amide nitrogen of an asparagine residue (Taborsky, 1974). The amino acid sequence of phosvitin is nearly complete (Taborsky, 1974), facilitating the interpretation of the data,

Department of Food Science and Technology, University of California, Davis, California 95616.