

## THE CAROTENOID AND PROVITAMIN A CONTENT OF THE WATERMELON

By L. ZECHMEISTER AND A. POLGÁR

(From the Gates and Crellin Laboratories of Chemistry,\* California Institute  
of Technology, Pasadena)

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10 years ago lycopene,  $C_{40}H_{56}$ , and carotene,  $C_{40}H_{56}$ , were isolated from the pulp of the European watermelon (*Citrullus vulgaris*, Schrad. = *Cucumis citrullus*, L.) by Zechmeister and Tuzson (1), and it was shown that the chief pigment, lycopene, is responsible for the red color. As the chromatographic method was not available at that time, no precise information as to the composition of the pigment was obtained. In some new experiments described below we have carried out a quantitative analysis of the components and have estimated the provitamin A content of the California watermelon. 1 kilo of the pulp examined contained 1.0 mg. of a complicated xanthophyll mixture, 6.1 mg. of lycopene, 0.06 mg. of  $\gamma$ -carotene, 0.16 mg. of unknown carotenoids (located in the column between  $\gamma$ - and  $\beta$ -carotene), 0.46 mg. of  $\beta$ -carotene, 0.01 mg. of  $\alpha$ -carotene. The figures include the fractions of lycopene, and  $\gamma$ - and  $\beta$ -carotene which underwent isomerization during the experimental procedure (2).

The colorimetric value of the total extract of 1 kilo of pulp corresponded to 7 to 8 mg. of "lycopene"; some samples were, however, considerably richer in pigment. Our material, picked in California in September, contained, according to the above figures, 0.5 mg. of provitamin A in 1 kilo of pulp, or about one-fifth to one-sixth of the daily  $\beta$ -carotene requirement of an adult person.

It is interesting to note that a considerable number of yellow and pink unidentified oxygen-containing carotenoids were found in minute quantities; *i.e.*, to the extent of about 0.01 mg. per kilo of pulp. Even with the use of chromatography 1000 or more kilos of melon would be needed for a satisfactory study of these

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pigments. One of them is spectroscopically identical with torulene, detected by Lederer in red yeast (3).

#### EXPERIMENTAL

##### *Methods*

The two parts of the chromatographic tubes were connected by a ground glass joint and the lower one was equipped with a perforated glass filter plate, as previously described (4).<sup>1</sup> All spectra were determined in an "evaluating grating" spectroscope as devised by Loewe and Schumm and manufactured by Zeiss (Jena). In accordance with a suggestion by Emerson and Fox (5), Jena colored optical filters No. BG-7 were adopted as light filters. For the quantitative estimation of the carotenoids a Pulfrich gradation photometer (Zeiss) was used, with light filter No. S-45 or S-47; the new photometric values given by Cholnoky (6) were used as a standard. Since no such data are available for  $\gamma$ -carotene and for torulene, an average of the colorimetric values of  $\beta$ -carotene and of lycopene in petroleum ether<sup>2</sup> is the basis of the calculations for the  $\gamma$  compound, and Cholnoky's capsorubin values are used for torulene.

##### *Color Analysis of Pulp*

The pulp of a 7 kilo melon (Klondike, 4.3 kilos without seeds) was mashed in a meat chopper and pressed in a hand press. The main pigment content was in the pulp; the small amount in the juice was in the floating red particles. After the addition of 0.05 volume of acetone, the liquid was kept in a narrow bottle overnight. A fine red material settled from which the clear liquid was cautiously decanted. The sediment was filtered off and the conic filter paper containing the insoluble material was dried at 40°. The pressed pulp was kept in methanol overnight and again pressed out. The slightly colored methyl alcohol was then added to the acetone-containing liquid mentioned above, and all the pigment was extracted by repeated shaking with a 1:1 mixture of benzene and petroleum ether. After evaporation the residue was dissolved in ether (Fraction I).

<sup>1</sup> This device is now manufactured by the Scientific Glass Apparatus Company, Bloomfield, New Jersey.

<sup>2</sup> The petroleum ether referred to throughout this paper is that having a boiling point of 60-70°.

The pressed material was dried at 40° (16 gm.) and combined with the dry sediment and this brownish red, hard material ground in a mill and ether percolated through it. The extraction was completed by using a smaller quantity of carbon disulfide which was collected separately and evaporated to dryness, and the residue dissolved in the ether extract and combined with Fraction I. The solution (450 cc.), containing practically all of the pigment present in the pulp, was saponified with 50 cc. of concentrated methanolic KOH overnight. After the addition of approximately 1 volume of water, the alkaline layer was discarded, the red solution washed three times with water, and the saponification repeated. The pigment solution was washed until free from alkali, dried over sodium sulfate, and evaporated to dryness under diminished pressure in a slow stream of CO<sub>2</sub>.

The dark residue was dissolved in 300 cc. of petroleum ether. The pigment content of this solution corresponded to 35 mg. of lycopene. It was chromatographed on calcium hydroxide (Shell Brand, chemical hydrated lime, 98.5 per cent; tube, 30 × 5.5 cm.) and developed first with petroleum ether, and later with benzene, until the following picture was obtained (the figures denote the height of the respective zones).

*Fraction A*—3 mm., brownish red; 2 mm., pale intermediate zone; 2 mm., red; 62 mm., bright red, the lower part stronger (lycopene); 11 mm., orange (neolycopene); 4 mm., light orange by-product of the isomerization.

*Fraction B*—10 mm., colorless; 15 mm., pale blurred orange-red; 18 mm., colorless; 20 mm., orange ( $\beta$ -carotene).

The column was cut into two parts; Fraction A was eluted with an alcohol-benzene mixture (1:4), and Fraction B with alcohol and petroleum ether (1:1). After the alcohol had been washed out of both solutions with water, Fraction A was dried, evaporated under diminished pressure, dissolved in petroleum ether, and chromatographed on calcium carbonate (Merck's heavy powder; tube as above). Fraction B was dried and rechromatographed on calcium hydroxide (Merck; tube 23 × 3 cm.). Both columns were developed with petroleum ether.

*Chromatogram A*—Xanthophylls: 5 mm., many thin yellow lines; 5 mm., several red and yellow lines; 2 mm., almost colorless; 5 mm., several pink and yellow lines.

Hydrocarbons: 60 mm., almost colorless; 95 mm., dark orange (lycopene); 4 mm., orange (neolycopene).

The lycopene and neolycopene layers contained 26.2 mg. of pigment. Maxima of lycopene, 547, 507.5, 475.5  $m\mu$ ; of neolycopene, 536, 498, 466  $m\mu$  (in carbon disulfide).

It was impossible to separate the individual xanthophylls, the total color intensity of which corresponded to 4.3 mg. of "lutein" This xanthophyll fraction gave, when rechromatographed on  $\text{CaCO}_3$ , at least twenty to twenty-five colored zones, some of which had been formed by isomerization. All were poorly separated. The majority of the yellow lines were located near the top, while in the next lower section pink and yellow lines were preponderant. The spectra of all these zones, taken in carbon disulfide, vary between 497.5, 466.5, 438  $m\mu$  and 568.5, 528, 493  $m\mu$ . The carotenoid showing the highest maxima, of which a few crystals were obtained, is spectroscopically identical with torulene; maxima in petroleum ether, 523.5, 491, 455.5  $m\mu$ ; in benzene, 539, 496, 459  $m\mu$ . The first bands are sharply bordered but narrow; the second ones appear broader and stronger. We believe that the quantity of this pigment is of the order of magnitude of 0.01 mg. in 4.3 kilos of the pulp.

*Chromatogram B (Spectra in  $\text{CS}_2$ )*—30 mm., colorless; 10 mm., dark orange ( $\gamma$ -carotene, 0.14 mg., 532.5, 494.5, 461.5, 430.5  $m\mu$ ); 3 mm., colorless; 15 mm., yellow (neo- $\gamma$ -carotene,<sup>3</sup> 0.15 mg., (527), 492, 458.5  $m\mu$ ); 15 mm., lemon-yellow (unknown, 0.07 mg., (525), 490.5, 458.5, 430  $m\mu$ ); 8 mm., colorless; 20 mm., orange (unknown, 0.62 mg., 512.5, 479.5, 452  $m\mu$ ); 3 mm., colorless; 15 mm., dark orange ( $\beta$ -carotene, 1.5 mg., 520, 484, 454  $m\mu$ ); 1 mm., colorless; 13 mm., light orange (neo- $\beta$ -carotene,<sup>4</sup> 0.5 mg., 513, 479  $m\mu$ ); 10 mm., blurred yellow (unknown, 0.04 mg.;  $\alpha$ -carotene?).

#### *Isolation of Lycopene and of $\beta$ -Carotene*

The preparation of the material and the extraction were carried out as described above, with the exception that the sediment was separated in the centrifuge. 83 kilos of watermelon (variety Rattlesnake) gave about 50 kilos of pulp, 490 gm. of dried brownish red powder, and finally 500 cc. of a dark petroleum ether solution which contained the total saponified pigment. Two chromatographic tubes (30  $\times$  7.5 cm.) were necessary. The

<sup>3</sup> This layer was identified as neo- $\gamma$ -carotene by partial conversion into  $\gamma$ -carotene on standing. We intend to describe the isomerization of this hydrocarbon later.

<sup>4</sup> Termed also pseudo- $\alpha$ -carotene.

sequence of the zones was like that of the first chromatogram described above, the separation, however, being much less clear, owing to the comparatively higher amount of pigment per unit weight of adsorbent.

*$\beta$ -Carotene*—The layer was cut out, eluted with an alcohol and petroleum ether mixture, rechromatographed, transferred into petroleum ether, and, after evaporation of the latter, crystallized from benzene after addition of methanol. The yield was 19 mg. of glittering plates. Maxima in carbon disulfide, 520, 485  $m\mu$ ; in petroleum ether, 485, 452.5  $m\mu$ . No separation from added  $\beta$ -carotene (from carrots) was obtained in the column. The mother liquor contained a considerable quantity of crystallizable sterols and other colorless substances, as well as 1.5 mg. of carotene.

*Lycopene*—The lycopene and the neolycopene layers were cut out together with all the layers above  $\beta$ -carotene. This section was rechromatographed on calcium carbonate (precipitated, McKesson) in seven portions; each time a  $30 \times 5.5$  cm. tube was used and the column washed with large quantities of petroleum ether, whereupon a sharp separation of the top layers from the lycopene layer resulted. The latter was cut out, eluted with alcohol and petroleum ether (1:1), and chromatographed on  $\text{CaCO}_3$ ; it now showed only negligible zones above the lycopene. After elution the main pigment was transferred into petroleum ether which was dried and evaporated. The residue was dissolved in the smallest possible amount of carbon disulfide, diluted with petroleum ether, and the pigment crystallized out by addition of absolute ethyl alcohol. The yield was 217 mg. of long red needles. From another variety of watermelon (Klondike) the corresponding yield was 188 mg. Maxima in carbon disulfide, 547, 507.5, 475.5  $m\mu$ ; in petroleum ether, 503.5, 473.5, 445  $m\mu$ . No separation from tomato lycopene could be obtained in the Tswett column.

#### SUMMARY

The carotenoids of watermelon pulp were chromatographed and estimated. The provitamin A content was about 0.5 mg. of  $\beta$ -carotene per kilo of pulp and is of the order of magnitude found by Munsell (7) in feeding experiments with rats.

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