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Carotene and Lycopene in Rose Hips and Other Fruits

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Rose hips have been previously reported to contain carotene (Kuhn & Grundmann, 1934; Wokes, Johnson, Organ & Jacoby, 1942) and lycopene (Karrer & Widmer, 1928; Escher, 1928), and they have also been found to possess considerable vitamin A activity (Svensson, 1936). Sufficient care has not always been taken to differentiate between the various carotenoid pigments. Moreover, data are needed on the amounts found in different species.

This paper provides such data by describing a simple method of estimating carotene and lycopene separately in the presence of other pigments, which has also given satisfactory results when applied to tomatoes and *Solanum Dulcamara* berries. The results on dried rose hip extract have been checked by a biological assay confirming the presence of vitamin A activity.

METHODS

Materials. Ripe rose hips of known species collected in the Durham area during October and November 1943 by Prof. Heslop Harrison, and in the Royal Botanic Gardens, Kew, during September 1943 by Dr R. Melville were posted immediately to King's Langley and stored in dry bottles in a refrigerator until examined; tests at intervals during the experimental period showed no loss of carotenoids under the given storage conditions. The samples of dehydrated rose hips were taken from large-scale batches prepared in the Ovaltine Laboratories in 1942-4 from locally grown hips, mainly *Rosa canina* and *R. dumetorum*. Ripe *Solanum Dulcamara* berries were collected in the King's Langley area during October 1943 and examined immediately. Ripe tomatoes grown out of doors in King's Langley in 1943 were preserved in jars by the usual method and when opened for examination 3 months later were in excellent condition.

Extraction of carotenoids. For this we have used an excellent unpublished method for extracting carotene from plant material devised by Dr Vernon Booth, to whom we are greatly indebted for advice and help. It was applied to rose hips as follows:

About 5 g. typical rose hips were selected and weighed. The flesh was dissected from seeds, stalks, etc., divided into two portions and weighed. Tests showed that no significant loss of moisture took place during the dissection, so that results with the flesh could be correlated with those from whole fruits. Each portion of the flesh (c. 1 g.) was ground with quartz powder and a mixture of acetone and petrol ether (2:3 by volume). The supernatant yellow solution was decanted into a separating funnel and the residue ground with more acetone-petrol ether mixture until no more colour was extracted. (With dried extracts and other dry material a few drops of distilled water were added to facilitate extraction.) The combined extracts were then washed with distilled water by a continuous flow apparatus, until the absence of striae as the drops of water fell through indicated that all acetone had been removed. Usually about a litre of water, passing through at the rate of about 2 drops per sec., was required to remove the acetone from 50 to 100 ml. of combined extract. The xanthophylls, which usually formed less than half of the total pigments, were removed by shaking two or three times with one-third volume of diacetone until practically no further colour was removed. The diacetone was then washed out with water.

Chromatographic separation. The adsorbent used in this investigation was Alocol brand colloidal aluminium hydroxide (A. Wander Ltd., King's Langley) activated by heating at 100° for a few minutes immediately before use. Its watery suspension had pH 7.4-7.5 as compared with 6.5-9.5 for other brands of alumina. The average particle size determined by means of haemocytometer was 25-100 μ , which is considerably higher than the 7 μ found by Zechmeister & Cholnoky (1941) for Merck alumina standardized by Brockmann's method. However, comparison with Merck and several other brands of alumina in use in this country at present has shown the Alocol after activation to be as efficient as any for separating carotene and lycopene. Recovery experiments with widely varying proportions of β -carotene and lycopene also gave satisfactory results. Details of this comparison will be published later.

The petrol ether extract, prepared as above, was percolated through a column of alumina (20 $\times$ 2 cm.). The pigments were adsorbed in a zone about 1 cm. deep and about

1 cm. from the top. The chromatogram was developed with petrol ether containing 1% acetone, when the yellowish orange carotene layer quickly separated from the reddish orange lycopene layer and, moving rapidly down the column, could be completely eluted. This usually left at the top of the column a narrow yellowish band possibly due to small amounts of kryptoxanthin. The lycopene was then eluted with benzene. The separate solutions of carotene and lycopene were stabilized with nitrogen, and stored in the dark in a refrigerator, under which conditions no appreciable loss occurred during several days. Throughout the extraction and separation of the pigments oxidation was prevented by keeping them moist with solvent and by screening them from light.

Spectrophotometric estimation of carotene and lycopene. This was done with a Hilger-Nutting constant deviation wave-length spectrophotometer calibrated for wave-length measurements against the emission spectrum of a helium lamp and for density measurements against a set of neutral glasses standardized by the National Physical Laboratory. The solutions, diluted if necessary with the respective solvents, were placed in 10 or 20 mm. cells of Hilger's type D, which can be readily filled and stoppered without inclusion of air bubbles in the body of the cell. The mean density was determined at 450 m μ for carotene in petrol ether (b.p. 40–60°) and 482 m μ for lycopene in benzene, using the cross-over technique of Wokes & Still (1942) which compensates for zero changes and other instrumental causes of error. Readings were taken at 10 m μ above and below the given wave-lengths to define the exact position of the maximum, and in a number of instances the whole curve was determined from 450 to 520 or 530 m μ . The results, summarized in Table 1, showed excellent agreement between carotene and lycopene separated from different sources and specimens of the pure pigments examined by ourselves and other workers.

Table 1. *Absorption data for carotene and lycopene*

Solvent	β -carotene		Lycopene	
	$\lambda_{\text{max.}}$ (m μ)	$E_{1\text{ cm.}}^{1\%}$	$\lambda_{\text{max.}}$ (m μ)	$E_{1\text{ cm.}}^{1\%}$
Petrol ether	450	2500	474*	2425
Benzene	463	2250	482	2025
Chloroform	463	2200*	480*	2000*

* Morton (1942).

Calculation of results. When calculating the concentration of carotene and of lycopene in our solutions we used the $E_{1\text{ cm.}}^{1\%}$ values given in Table 1. The value of 2025 for lycopene in benzene was determined as follows. Lycopene was extracted from *Solanum Dulcamara* berries by the above method, and separated by chromatography from carotene and all other pigments. Solutions of the purified lycopene were prepared in chloroform, benzene and petrol ether (b.p. 40–60°) and their respective densities determined. From these densities were calculated the values for $E_{1\text{ cm.}}^{1\%}$ in benzene and petrol ether, the value given by Morton (1942) for lycopene in chloroform being used as a basis. The benzene solution of the lycopene gave max. 482 m μ (between max. 486 m μ found by Zechmeister & Tuzson (1938) and max. 480 m μ found by Sivadjian (1938)) and the petrol ether solution gave max. 473 m μ , in good agreement with max. 474 m μ (Morton, 1942). The same technique

applied to β -carotene in benzene gave $E_{1\text{ cm.}}^{1\%}$ of 2250 at max. 463 m μ , differing by less than 2% from the value of 2280 at max. 464 m μ (Morton, 1943). Hence the error introduced by using the value 2025 for lycopene was probably less than 2%, of which only part could be attributed to the difference of 2–4 m μ in position of maxima as observed by ourselves and other workers, since the absorption curves for lycopene and carotene in Fig. 1 show fairly broad peaks at the above maxima.

Fig. 1. Absorption curves for β -carotene (specimen also tested by R. A. Morton) and for lycopene extracted from *Solanum Dulcamara* berries. β -carotene in petrol ether + 1% acetone $\bigcirc$ — $\bigcirc$. Lycopene in benzene $\times$ — $\times$.

Sampling errors. These form the chief part of the experimental error. The average coefficient of variation for estimations on separate hips was 36. This was reduced to 15.7 by bulking 5 g. of hips comprising 3–12 hips, which after removal of seeds, etc., yielded 2–3 g. flesh. The average coefficient of variation between different 1 g. samples on the same lot of flesh from 5 g. portions of hips was only 5.1, showing that the method of preparing the samples had produced a much more uniform material than the original rose hip population. However, when dehydrated extracts of rose hips were made, the coefficient of variation between single assays on this material was still lower, showing that still greater uniformity had been obtained. Assuming that the experimental error was responsible for a coefficient of variation of not more than 3%, and deducting this from the average coefficient of variation of 36 for separate hips, there is left at least 33% as a measure of the variation to be expected from one rose hip to another.

RESULTS

Known species of rose hips. These are summarized in Tables 2 and 3 in order of decreasing carotene content. Each result is the mean of at least two assays in which the average error was as described above. The carotene content of the Durham samples was 74–187 $\mu\text{g./g.}$, and of the Kew samples 41–671 $\mu\text{g./g.}$ The lycopene content was usually higher than the carotene content, except in one or two

Table 2. *Carotene and lycopene in flesh of fresh rose hips from Durham*

	Carotene ($\mu\text{g./g.}$)	Lycopene ($\mu\text{g./g.}$)
Hybrid between <i>R. dumetorum</i> and <i>R. mollis</i>	187	276
<i>R. dumetorum</i> var. <i>Gabrielis</i>	161	232
<i>R. dumalis</i> var. <i>Reuteri</i>	149	532
<i>R. dumalis</i> var. <i>subcristata</i>	148	834
<i>R. canina</i> var. <i>sarmentacea</i>	146	268
<i>R. tomentosa</i> var. <i>soabruscula</i>	142	141
<i>R. obtusifolia</i> var. <i>sclerophylla</i>	119	314
<i>R. dumalis</i> var. <i>subcanina</i>	118	229
<i>R. coriifolia</i> var. <i>frutetorum</i>	113	246
<i>R. canina</i> var. <i>vinacea</i>	98	223
<i>R. dumetorum</i> var. <i>pseudoincerta</i>	74	101

Note. We are informed by Prof. Heslop Harrison that in following the International Rules of Botanical Nomenclature, *R. dumalis* is the correct name to use for the species described as *R. glauca* or *R. Afzeliana* in Wolley-Dod's *Revision of the British Roses* (1930-1), the varietal names being the same except for var. *subcristata*, which is var. *glaucohylla* W.-Dod. The use of *R. dumalis* in this sense necessitates the change of *R. canina* var. *dumalis* to *R. canina* var. *sarmentacea*.

Table 3. *Carotene and lycopene in flesh of fresh rose hips from Kew*

	Carotene ($\mu\text{g./g.}$)	Lycopene ($\mu\text{g./g.}$)
<i>R. centifolia</i>	671	206
<i>R. Davidii</i>	242	657
<i>R. carolina</i> var. <i>Nuttalliana</i>	233	598
<i>R. virginiana</i>	230	327
<i>R. moschata</i>	210	147
<i>R. calocarpa</i>	175	150
<i>R. Wilsoni</i>	169	367
<i>R. agrestis</i>	123	537
<i>R. Helenae</i>	108	94
<i>R. canina</i> var. <i>stenocarpa</i>	100	204
<i>R. Sabini</i>	93	238
<i>R. rubiginosa</i>	74	595
<i>R. mollis</i>	63	283
<i>R. spinosissima</i>	50	146
<i>R. coriifolia</i>	41	354

species. The ratio between carotene and lycopene in a given species did not vary in duplicates by more than experimental error. The result of $187 \pm 6 \mu\text{g./g.}$ for carotene in the hybrid of *Rosa mollis* and *R. dumetorum* was significantly higher than the results of 74 ± 1 for *R. dumetorum* hips collected at the same time in the same area, and higher than the result of 63 ± 5.0 for *R. mollis* hips from Kew, although not much higher than the result of 161 ± 11.2 for *R. dumetorum* var. *Gabrielis* hips from the Durham area. It should be noted that the hybrid was almost sterile, yielding less than a dozen hips on the whole bush.

Effect of latitude. Pyke & Melville (1942) found the vitamin C content of rose hips to be related to the latitude in which they are indigenous, the higher contents being found in higher latitudes. We did not find any positive relation between carotene content and latitude in native species showing

Table 4. *Distribution of rose hips and their vitamin A and C values*

	Vitamin C* (mg./100 g.)	Carotene ($\mu\text{g./g.}$)
<i>R. mollis</i>	1260	63
<i>R. coriifolia</i>	1080	77
<i>R. rubiginosa</i>	810	74
<i>R. tomentosa</i>	690	142
<i>R. dumetorum</i>	590	118
<i>R. canina</i>	550	123
<i>R. agrestis</i>	460	123
<i>R. obtusifolia</i>	420	119
<i>R. spinosissima</i>	340	50

* Pyke & Melville (1942).

marked differences in their vitamin C content (see Table 4). In fact our data suggested a negative correlation but were insufficient to establish this significantly.

Comparison of carotene content with vitamin A activity. For this comparison it was thought desirable to obtain material of greater stability and more uniform composition than could be found in fresh rose hips. Dried extracts were therefore prepared from fresh ripe hips by extraction with boiling water and evaporation of the extract *in vacuo*, with precautions to avoid loss by oxidation. Tests showed that not more than 20 % of the vitamin C had been lost during the preparation of the extracts, which were found to be rich sources of vitamins C and P as well as containing considerable amounts of carotene. A biological assay of a sample of one of these dried extracts, after it had been stored about 9 months in powdered form in air at room temperature, was carried out in the Pharmaceutical Society's laboratories and showed it to have vitamin A activity equivalent to 44 i.u./g. (23-86 for $P = 0.95$). Chemical estimation of the carotene content before and after the biological test showed a gradual loss of carotene (from 45 to 16 i.u./g. in 14 months) and the carotene content at the time of the biological test was only about 60 % of the amount shown by the biological test, if one assumes that 1 $\mu\text{g.}$ carotene = 1.66 i.u. vitamin A. This rate of loss can be greatly reduced by storing the extracts in the form of compressed tablets. Taking into consideration the error of the biological method as well as other possible sources of error, this difference was not highly significant. However, since the biological result was higher than expected on the chemical assay indicated, it was clear that the carotene estimation did not provide too high an indication of the vitamin A value, and that the chromatographic separation of lycopene from carotene was complete.

Results on other materials. The method, having given satisfactory results with rose hips in which the L/C (lycopene/carotene) ratio ranged from 0.3 to 8.7, was then applied to other materials in which this ratio varied still more widely, e.g. *Solanum*

Dulcamara berries with L/C ratio of 17.2, dehydrated carrots rich in carotene and containing practically no lycopene, and tomatoes, which have in the past been given exaggerated vitamin A values because of failure to differentiate efficiently between carotene and lycopene. The results, summarized in Table 5, showed satisfactory separation of the carotene from the lycopene and were on the whole in good agreement with those obtained by other workers who also separated the two pigments satisfactorily (Kuhn & Grundmann, 1934).

Table 5. Carotene and lycopene contents of different materials

	Carotene ($\mu\text{g./g.}$)	Lycopene ($\mu\text{g./g.}$)
<i>R. centifolia</i> hips	671	206
Kew rose hips, 15 varieties, mean	172	327
Durham rose hips, 11 varieties, mean	132	309
Tomato skins, cooked, mean	73	416
<i>R. coriifolia</i> hips	41	354
Tomato flesh	7	33
<i>Solanum Dulcamara</i> berries	6	103
Results by other workers:		
Kuhn & Grundmann (1934):		
Tomatoes	7	79
<i>R. canina</i> hips	255	160
<i>R. rubiginosa</i> hips	230	275
Smith (1936):		
Tomato skins	2652-8862	—

In the skins of cooked tomatoes the carotene content of a number of samples was very much lower than that found by Smith (1936) in the skins of American tomatoes. The lycopene/carotene ratio in the skins was, however, similar to that found in tomato flesh by ourselves and by Kuhn & Grundmann (1934).

DISCUSSION

The estimation of carotene in materials containing lycopene presents difficulties. Failure to achieve efficient separation may lead to exaggerated claims for the vitamin A value of certain foods (e.g. tomatoes) if these claims are based on carotene estimations. Moreover, during the extraction of the pigments changes may occur through isomerization or oxidation. These difficulties have been overcome by using (a) extraction methods avoiding the use of heat and requiring minimum of exposure to air and light; (b) chromatographic separation with a

specialty prepared adsorbent of proved efficiency; (c) immediate spectrophotometric estimation of the pigments.

These precautions have enabled us to obtain reasonably accurate results on the different species of rose hips as shown by the coefficient of variation of 5.1 for different samples from the same batch of minced rose hip flesh.

It should be emphasized that this degree of accuracy is applicable only to the experimental material as we obtained it, and does not take into consideration variations caused by habitat, climate and other factors. However, since the average figure for all the Kew samples varied by less than 30 % from the average figure for all the Durham samples, the differences in conditions in these two areas could not account for the much wider variation in carotene and lycopene content in different species. Moreover, in a series of dried extracts made mainly from *Rosa canina* and *R. dumetorum* hips collected in the King's Langley area, there was less than 30 % deviation from one batch to another and from 1942 to 1943 seasons. Hence there is little doubt that ripe rose hips in general contain considerable amounts of carotene, and that some species contain much more than others.

SUMMARY

1. A method is described for extracting, separating, and estimating carotene and lycopene from plant materials, which minimizes isomerization and oxidative changes.

2. Applied to the flesh of ripe rose hips, this method showed carotene contents of 41-671 $\mu\text{g./g.}$ and lycopene contents of 94-834 $\mu\text{g./g.}$ in 26 distinct species and hybrids.

3. The method also gave satisfactory results with dried rose hip extracts, *Solanum Dulcamara* berries, tomatoes and other sources of carotenoids. The vitamin A value of a sample of dried rose hip extract as determined by biological assay was at least as high as was indicated by our chemical estimations of the carotene.

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