

Analysis of 100 gm. of Fruit

Common Name	Botanical Name	Proteins gm.	Sugars gm.	Phosphorus gm.	Calcium gm.	Vitamin B ₁ mgm.	Vitamin C mgm.
1. Chiku	<i>Sapota zapotilla</i>	0.938	9.21	0.012	0.019	0.078	
2. Orange	<i>Citrus aurantium</i>	0.628	8.31	0.013	0.013	0.108	53.5
3. Figs	<i>Ficus carica</i>	1.114	9.07	0.024	0.052	0.042	
4. Guava	<i>Psidium guajava</i>	1.39	9.44	0.028	0.109	0.045	
5. Plantain—Velchi ..	<i>Musa sapientum</i>	1.15	18.33	0.02	0.003	0.138	0.82
6. Plantain—Green Skin	“ ”	1.07	17.83	0.039	0.003	0.132	
7. Plantain—Rasbali .	“ ”	1.11	18.07	0.027	0.006	0.126	
8. Apple Red ..	<i>Pyrus malus</i>	0.25	10.48	0.022	0.007	0.099	1.08
9. Apple—Yellow green	“ ”	0.242	10.50	0.022	0.007	0.090	1.24
10. Grapes—Yellow ..	<i>Vitis vinifera</i>	0.54	14.69	0.016	0.24	0.036	

which has 30 per cent. water-insoluble (acid-soluble) when analysed with skin on, the rest being water-soluble. The amounts of vitamins present are fairly high especially B₁ in plantain, apple and sapota.

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1. Rege and Devadatta, *J. Univ. Bomb.*, 1941, **10**, 3, B, 74. 2. *Nature*, 1933, **15**, 132. 3. Tauber, H., *Mikrochem. Acta*, 1938, 108.

A CHROMATOGRAPHIC ADSORPTION METHOD FOR THE ESTIMATION OF THE PROVITAMIN A CONTENT OF FOODSTUFFS

RECENT work¹ from this laboratory has shown that the petroleum ether-methyl alcohol phase partition for the separation of carotene from xanthophyll is unsatisfactory because some coloured but biologically inactive degradation products also remain in the epiphasic layer and are, therefore, estimated as carotene. Errors due to the presence of such non-carotene pigments have been found to be rather high in the case of stored foodstuffs like cereals, pulses and condiments. Further, the inactive isomer, lycopene, is likely to be estimated as carotene while no account is taken of the fact that β -carotene is biologically twice as potent as any of the other pro-vitamins A. A correct estimation of the vitamin A activity of any vegetable material can be made only by determining the amounts of the different provitamins present and employing the formula: Vitamin A activity in International Units =

$$\frac{\mu\text{g of } \beta\text{-carotene}}{0.6} + \frac{\mu\text{g of other provitamins}}{1.2}$$

Chromatographic adsorption which offers the only means of separating these pigment

mixtures has not been used as a routine method of estimation since the pigments may be lost to the extent of 10 to 20 per cent. due to incomplete elution from the adsorbent. However, by the application of the chromatographic adsorption in two stages and by a choice of suitable adsorbents, it has been possible to estimate firstly, the total carotene (including lycopene, if present, but no artifacts) and then the relative proportions of the constituent pigments. After repeated trials with a number of substances, dicalcium phosphate prepared according to Moore² was found to be satisfactory for the first chromatography. Xanthophylls and artifacts are strongly adsorbed by it, while carotene and lycopene pass down practically unadsorbed. Cryptoxanthin is only weakly adsorbed and, therefore, it can be collected separately by further developing the chromatogram. Recovery experiments with pure β -carotene solutions have shown that the losses are never more than 2 per cent. with a properly prepared adsorbent column.

The carotene solution thus obtained is concentrated to a small volume and chromatographed over a column of Brockmann's alumina. The bands are eluted separately and the different pigments identified and their concentrations determined. The eluates may account for a recovery of about 85 per cent. only but since the three common hydrocarbon pigments— β -carotene, α -carotene and lycopene—differ very slightly in their adsorbabilities, it may be reasonably assumed that the losses would be proportionate. Using this proportion the quantities of the individual pigments present in the original carotene extract (first chromatography) are calculated.

A number of foodstuffs have been assayed for their provitamin A content employing the extraction procedure described in an earlier paper³ in conjunction with the adsorption technique described above. All the estimations were on petroleum ether (b.p. 60-75° C.) solutions, taking readings at three wavelengths (450, 470 and 480 $m\mu$) in a visual spectrophotometer and employing extinction coefficients derived from a sample of pure β -carotene isolated from Badami mango fruit.⁴

Provitamin A content in 100 grams of material

By the authors

Name of the Material	Health Bulletin 1 μ g = 1 I.U.	Phase partition method μ g. "Carotene"	Chromatographic method μ g. "Carotene"	Composition of the "Carotene" (per cent.)	Vitamin A acti- vity in Int. Units (Calculated)
Papaya fruit	2020	—	1280	C 90; β -10	1170
Cashew seed	1570	230	100	L 55; β -45	73
Dry chillies	578	11200	6230	β -71; α -26	8820
Gingelly seeds	100	21.0	12.0	—	< 20
Whole wheat	108	21.0	(?) 6.0	—	< 10
Horse gram	119	74	16	Mostly β -	27
Mango	—	3580	2150	L(?) 92; β -8	290
Ferrogreen seeds	160	660	260	β -92; ? 8	420

L = lycopene;

C = cryptoxanthin;

 α = α -carotene; β = β -carotene

? = doubtful identity.

A few typical results are presented in the above table. For the sake of comparison, values for the carotene content given in Health Bulletin No. 23 (Third Edition) of the Government of India, are also included in the table; these are mostly obtained from the work of De and co-workers.⁵ The figures clearly show that, apart from varietal differences and individual variations, a large proportion of the pigment present in stored foodstuffs and estimated as carotene by the phase separation method may be actually of a non-carotene nature. Further, the results indicate the need for a thorough re-investigation of the common food materials employing these improved methods.

Finally, a word of caution is necessary with regard to the first chromatography. There are many variables in the experimental procedure—the adsorptive power and particle size of the dicalcium phosphate, size of the column and the method of packing, vacuum applied for packing and during the experiment, etc.—and unless special care is taken, considerable errors may be introduced into the determinations. At the outset, all these experimental conditions should be standardised to give quantitative recoveries of carotene and the details strictly adhered to subsequently. It is further recommended that each lot of adsorbent be tested to give a proper performance before making use of it for estimations.

In the case of leafy vegetables and similar rich sources, saponification of the pigment extract may be omitted but it is essential in the case of poorer materials since the presence of more than 25 mg. of oil in the extract interferes with the adsorption of some of the non-carotene pigments while more than 150 mg. of oil is definitely objectionable.

Full details of the method and analytical data on the provitamin A content of a num-

number of foodstuffs under investigation, will be published elsewhere.

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THE EFFECT OF NUTRITION AND CLIMATE ON RAT LEPROSY

CLINICAL experience of leprosy has given a universal impression all over the world that it is a "poverty disease". It means that more than infection predisposition is a more vital factor and this lies in some defective nutrition. To solve this mystery attempts have been made and are still being continued to trace the nutritive factor to some vitamin deficiency particularly to that of vitamin A. Apart from this, regional distribution of leprosy has been well recognised as an unsolved problem. There may be a village where leprosy incidence may be ten per hundred or even more whereas in the whole geographical province where food and race are not apparently different it may not be more than ten in a thousand of population.

Unfortunately no laboratory animal has data on the provitamin A content of a