

I. The Carotene Content of Some South African Feeds.

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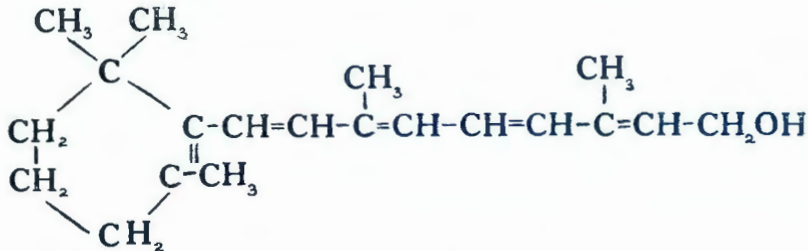
GENERAL OUTLINE.

CAROTENE, belonging to the group of carotinoids—yellow, fat-soluble pigments, of which there are no less than twelve different ones known—has the most extensive occurrence of all natural pigments. Three of the carotinoids, namely, the well-known Lycopin—characteristic pigment of the tomato—and the two isomers, α -Carotene and β -Carotene, each having the general formula $C_{40}H_{56}$, are in character hydrocarbons. The other carotinoids, which are oxygen-containing derivatives of Carotene or Lycopin, have the characteristics of organic acids, with a molecular formula of $C_{35}H_{50}O_3$ —commonly known as “Phytoxanthines”.

The study of the Carotinoids was first started in 1831 by Wackenroder and later in 1837 by Berzilius. Working on leaf-pigments, they discovered that the pigments were closely associated with fats and oils—hence the name Xanthophyll was given to the yellow pigment of leaves by Willstätter (1910). Other workers also gave the carotinoids their attention: Karrer and Solaman (1927) isolated Crocetin ($C_{20}H_{24}O_4$); Zechmeister and Cholnoky (1927) isolated Capsanthin; Karrer and co-workers (1929, 1930) isolated Zeaxanthin; Kuhn and co-workers (1931) isolated Violaxanthin and Zoraxanthin.

All the carotinoids are practically insoluble in water, and easily soluble in fats and oils. They have the characteristic of fat-pigments (Lipochromes) and are therefore fairly common in nature. As regards solubility in the ordinary organic solvents, there are two distinct groups of carotinoids, namely the hydrocarbons (Carotene and Lycopin)—soluble in petroleum ether, benzene, but insoluble in methyl- and ethyl alcohol. The phytoxanthins (Leaf-xanthophyll, Zeaxanthin and others) are insoluble in petroleum-ether and benzene, but soluble in methyl- and ethyl alcohol. Hence the carotinoids can be fractionated into Carotene and Lycopin on the one hand and the phytoxanthins on the other hand, as was shown by Willstätter. Most of the carotinoids are easily oxidised and become bleached under influence of oxygen. Crocetin, Bixin and Azafrin are exceptions (Karrer). The rate of oxidation depends largely on the state of purity of the pigments. Thus a very pure carotene will only absorb oxygen after ten or more days, whereas the

He furthermore considers β -Carotene the precursor of Vitamin A, which is formed by hydrolysis:—



Apart from cod-liver oil and other liver oils as a source of Vitamin A in biological material, the Carotene of the plant is the natural source of Vitamin A for herbivora. Numerous investigations prove that animals on a diet deficient in Carotene show A-avitaminosis and the young that are dependent on the milk supply of the mother, show stunted growth.

Plants vary in their Carotene content according to species, stage of growth, and portion of plant selected. Lucerne in young, green stage is a rich source of Carotene for Vitamin A, becoming poorer as the plant matures; the leaves are richer than the flowers, stems and seeds. In some plants at the mature stage there is a serious depletion of Carotene; even Carotene-rich plants show only a very small amount at maturity.

Furthermore, hays may also be low in Carotene content; the retention and preservation thereof depending to a great extent on the process of drying and curing. Carotene is easily oxidised by enzymes contained in the plant material, hastening deterioration, as shown by S. Hauge (1935). In the drying and curing of lucerne for hay, the slower the process, the better the oxidation of Carotene, which, therefore, results in a loss of Carotene to animals fed on such hays.

Mechanical drying and curing of lucerne for hay, apart from being time-saving, is far superior to sun drying from the point of view of Carotene-preservation.

In South Africa, where sun drying is the main process in use, loss of Vitamin A may prove to be a matter for consideration, as most of our sun-dried hays are low in Carotene.

METHOD OF ANALYSIS OF FEED FOR CAROTENE.

Biological tests with Carotene have often proved unsatisfactory, reproducible results being often unobtainable; a further factor is time.

It has been found by Lathbury and Greenwood (1934) that some oils are beneficial and others not,—causing a loss of Carotene in the latter case,—when used as solvents. Coconut oil is a better solvent, than for example arachis oil. Hence difficulties in biological experimental work have been encountered.

Lately chemical methods for the analysis of material for Carotene have been developed. Guilbert (1934) in working on this subject, employs a colorimetric method for the quantitative expression of Carotene, and the colorimetric work is based on the use of the Colour Standard (International Dye Standard) of Sprague (1928).

In the analysis of various feeds and fodder in this laboratory, the method as given by Guilbert was closely followed. Duplicates of a satisfactory value are obtainable.

The Method of Extraction suggested by Guilbert, produced here :

1-5 gms. of the sample, in finely cut state, was weighed out, transferred to an Erlenmeyer flask and 20 c.c. of freshly prepared saturated solution of potassium hydroxide in ethyl alcohol were added for each gram of sample taken. The flask was fitted to a reflux condenser and the contents gently boiled on a steambath for 30 minutes. Fats and chlorophylls became saponified. The contents of the flask were cooled, 50-100 c.c. ethyl ether added, shaken for a few minutes, the sediment allowed to settle and the ether-alcohol mixture decanted into a large separatory funnel. The flask was washed with small quantities of ether and these were added to the contents of the funnel. The process was repeated until fresh ether when used for rinsing, was colourless (usually 200-250 c.c. ether were necessary for each extraction).

About 100 c.c. (or more) of distilled water were poured down the sides of the funnel through the ether-alcohol solution. The flavones separated and could be drawn off. The solution in funnel was repeatedly washed, drawing off the bottom layer each time. The waste was tested with phenolphthalein to be sure that all alkali had been washed out of the ether-solution.

The ether solution, which contained the yellow pigments Carotene and Xanthophyll, was transferred to a flask and all traces of ether expelled.

The residue was then taken up in 30-40 c.c. petroleum-ether and transferred to a separatory funnel. The Xanthophyll was extracted from the ether solution and separated from Carotene by first shaking with 85 per cent.—and finally with 90 per cent. methyl-alcohol. The lower layer (methyl-alcohol) containing the dissolved Xanthophyll, was separated from the ether solution containing the dissolved Carotene. After 5-6 washings of the ether layer, the methyl-alcohol layer was found to be clear; the last traces of methyl alcohol were rinsed out with small quantities of distilled water.

The ether solution was next dried in contact with about 5 gms. of anhydrous sodium sulphate; it was then poured off, and the salt washed with fresh petroleum ether, until clear—the washings added to the rest of the Carotene-ether solution. The ether solution was poured into a measuring flask, and made up to volume (namely 50 c.c. mark) with petroleum ether. The volume was noted.

The flask was next well shaken and the strength of the Carotene-colour compared against a dye standard in the colorimeter.

A stock solution of this dye standard contained:

Naphthol yellow, 3·06 grams;
Orange G. Crystals, 0·45 grams.

The dyes were dissolved in 1,000 c.c. distilled water. The solution is stable and will keep well in a stoppered flask in the dark.

The standard for the colorimetric work was prepared from the above stock solution, by diluting 50 c.c. of the stock solution to 1,000 c.c.—this colour strength of Dye is equivalent to 2·7 mgm. Carotene (Guilbert).

Calculations:

$$\frac{\text{Depth of Standard}}{\text{Colorimetric Reading}} \times \frac{100}{\text{Grams Sample}} \times \frac{\text{Total volume of unknown}}{1,000}$$

× Value of Standard in mgm. Carotene (2·7) per 1,000 c.c.
= Mgm. Carotene per 100 grams Sample.

TABLE A.

RELATIVE β -CAROTENE VALUES OF VARIOUS FEEDS.

(Value of Dye Standard used = 2·7 mgm. per cent. Carotene.)

Description of Sample.	Weight of Sample. (Wet basis) grams.	Moisture Content %	Mgm. % Carotene on Abs. dry basis.	Mgm. % Carotene on fresh basis.
<i>Fresh, young, green Lucerne</i> (whole plant, leaves and stalks).....	15	77·5	40·0	9·0
<i>Fresh, green, Sudan Grass</i> , late stage—(whole plant, leaves and stalks).....	15	79·1	13·5	2·8
<i>Fresh, green Maize plants</i> , late stage—(whole plants, leaves and stalks).....	30	74·0	3·4	0·9
<i>Fresh, green Barley plants</i> (whole plants, leaves and stalks).....	25	79·0	17·1	3·5
<i>Mature grass cut for hay</i> (Armoedsvlakte—Camp A—June, 1935). (Sun dried)....	100	—	—	0·62
<i>Mature Grass cut for hay</i> (Armoedsvlakte—Camp B—June, 1935. (Sun dried)....	100	—	—	0·09

CAROTENE CONTENT OF SOME S.A. FEEDS.

TABLE B.

RELATIVE β -CAROTENE VALUES OF FODDER-HAYS.
(Value of Dye-Standard for Carotene = 2.7 mgm. per cent.)

Description of Sample.	Weight of Sample. (Air dry basis). grams.	Moisture Content. %	Mgm. % Carotene on Abs. dry basis.	Mgm. % Carotene on natural basis.
<i>Lucerne Hay</i> , sun-dried, well-cured (Onderstepoort).....	30	7.6	0.92	0.80
<i>Teff Hay</i> , well-cured (Onderstepoort)....	20.4	1.0	2.50	2.30
<i>Mature Grass Hay</i> , sun dried, well-cured (Onderstepoort).....	100	8.3	± 0.02	± 0.018
<i>Yellow Maize Seed</i> —Finely ground.....	100	9.4	0.74	0.67
<i>White Maize Seed</i> —Finely ground.....	100	—	—	trace
<i>Samp</i> —Finely ground (maize endosperm).	100	—	—	negative
<i>Pig Feed</i> —Mixture of samp and meatmeal (9:1).....	100	—	—	0.047
<i>Maize Meal</i> (yellow).....	100	—	—	0.25
<i>Bran</i> (Wheaten).....	100	—	—	<0.01
<i>Barley Meal</i>	100	—	—	<0.01

TABLE C.

RELATIVE VALUES FOR CAROTENE OF VELD GRASS SAMPLES TAKEN AT DIFFERENT SEASONS FOR TWO SUCCESSIVE YEARS (ARMODSVLAKTE).

Sample. Month of Collection.)	Season.	Mgm. % Carotene (on Abs. dry basis).	Mgm. % Carotene (on Air dry basis).
SAMPLE I..... <i>October, 1933</i>	Spring	0.11	0.11
SAMPLE II..... <i>January, 1934</i>	Summer	1.85	1.71
SAMPLE III..... <i>April, 1934</i>	Autumn	0.98	0.92
SAMPLE IV..... <i>July, 1934</i>	Winter	0.26	0.24
SAMPLE V..... <i>October, 1934</i>	Spring	0.61	0.57
SAMPLE VI..... <i>January, 1935</i>	Summer	2.64	2.45
SAMPLE VII..... <i>April, 1935</i>	Autumn	4.66	4.32
SAMPLE VIII..... <i>July, 1935</i>	Winter	0.23	0.22

DISCUSSION.

Samples of some of the feed and fodder materials fed to farm animals at Onderstepoort, were analysed for Carotene.

It is evident from Table A that lucerne in young stage of growth is a rich source of carotene.

The green Sudan grass and green Barley, though cut at a fairly late stage of growth (seeding stage) still had appreciable amounts of Carotene. The low Carotene content of the sample of maize plant was probably due to the late stage of growth of this material.

In the case of grass hay the Carotene content ranged from 0.1 to 0.6 mgm. per 100 grams dry material. Hays, in general (Table B) are variable in their Carotene content, the amounts depending on the process of drying and curing. Teff hay gave as much as 2.3 mgm. Carotene per 100 gms. dry material. Probably this teff was cut for hay at a favourable growth-period and was dried and cured under conditions which were favourable for the preservation of its Carotene.

Well-cured, sun-dried lucerne hay was low, probably due to considerable loss of Carotene during the drying and curing processes, as was found to be the case by S. Hauge (1935) in experimental work.

Some mature grass when cut for hay proved to be as low as 0.02 mgm. Carotene per 100 gms. dry material. This hay, even if fed liberally cannot serve as an adequate source of Carotene, if no green material or other food, containing Carotene is given; animals will in time suffer from the effects of Vitamin A deficiency.

Of the maize (seed), the yellow variety, though low in Carotene, was much higher than the white variety.

Analysis of veld grass samples from Armoedsvlakte, Bechuanaland for different seasons of two successive years are tabulated in Table C. For a greater part of the year the natural veld-grass at Armoedsvlakte is apparently low in Carotene. In 1934-35 this was very pronounced during the dry period (winter and spring); in the summer, when copious rains fell (summer-rainfall area) there was new growth and the Carotene of samples taken during that period showed a gradual increase, reaching its maximum in Autumn. Towards winter there was a decline which might continue into Spring or until new growth appeared.

As Carotene is the precursor of Vitamin A, which is essential for the growing animal, as also for normal reproduction, good health and high vitality, it would appear that the danger of an A. avitaminosis during the dry periods of the year in areas like Armoedsvlakte, which are common in other parts of the Union, cannot be excluded. From work which is proceeding it may even be tentatively concluded that such deficiency does exist at times for normal growth and maintenance. Furthermore, hays cured under prevailing conditions and fed back to animals during such period of food scarcity might not contain adequate quantities of Carotene to make good the deficiency in the natural pastures.

The Vitamin A requirements of animals, except pigs—which according to Dunlop (1935) require daily per 100 lb. live-weight approximately 4 mgm. Carotene, are uncertain, but with data that winter pastures in summer-rainfall area are usually low in Carotene, the problem of A-avitaminosis during dry periods, especially under ranching conditions, is well worth further investigation especially in the light of work of Guilbert and Hart. Such work has been started and will be reported on in due course.

SUMMARY.

1. Details are given of the method employed at this Institute for the determination of Carotene in some animal foodstuffs.

CAROTENE CONTENT OF SOME S.A. FEEDS.

2. The Carotene content of pasture diminishes rapidly as the pasture matures and becomes dry during winter or during dry periods of drought.

3. The Carotene content of the Cereals included in the determinations is low, yellow maize being the highest and the sample in question containing 0.74 mgms. Carotene per 100 gms. dry material.

4. The existence of an A-avitaminosis in stock entirely dependent on natural pastures during periods, when only dry pasture exists, is discussed.

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