
THE RELATION BETWEEN SO CALLED CRUDE AND TRUE CAROTENE OF NATIVE GRASS HAY

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The value of green plants and certain plant products as sources of vitamin A for animals and human beings has been traced to the presence in these materials of one or more naturally occurring yellow pigments. Two of the most important of these pigments having vitamin A activity are carotene, which is widely distributed among plants, and cryptoxanthin, which is found in yellow corn. Some of the more common yellow pigments accompanying carotene and closely related to it chemically, but which have no vitamin A value, are xanthophyll (lutein) which is abundant in green grass, lycopene which is present in tomatoes, and zeaxanthin which is found in egg yolk and leaves. All of these pigments belong to the general class of compounds known as carotenoids. The quantitative separation of carotene from these and other associated pigments as a means of estimating the vitamin A value of plant products has been one of the major analytical problems in biochemistry.

¹Hanford, U. S. Patent 2, 396, 786 C. A. 49:3630 (1945).

²Kharasch and Coworkers, *Science* 102:128 (1945); *J. Am. Chem. Soc.* 67:1626-1694 (1945); 68:154 (1946); 69:1100-1110 (1947) and subsequent articles.

In the determination of carotene in dry forages, the sample is saponified with alcoholic KOH and the resulting mixture extracted with Skellysolve B. By dilution of the extract with water, two liquid layers are formed. The lower layer, which contains the chlorophylls and other pigments, is drawn off and discarded. The upper layer, containing a mixture of carotene, xanthophyll and other yellow pigments, is washed with 90% methyl alcohol to remove xanthophyll and a large proportion of the yellow pigments other than carotene. After further washing and drying, the total yellow color of the Skellysolve solution is determined colorimetrically and reported as crude carotene (Peterson, 1941).

For the determination of so called true carotene the Skellysolve extract is further purified by pouring it through an adsorption column of magnesium oxide and Hyflo Supercell as described by Wall and Kelly (1943). Carotene is eluted from the column with a 4 per cent solution of acetone in Skellysolve B. Many of the colored impurities in the crude carotene extract not removed by previous extraction with methyl alcohol are retained in the column. The color of the acetone-Skellysolve B solution is taken as a measure of the true carotene of the sample.

Since the results of crude carotene determinations are easily reproducible whereas errors are frequently encountered in the determination of true carotene, the former method is sometimes preferred. Both methods give relative but not absolute values.

During the past three years the true carotene content was determined on 140 samples of native grass hay and the values plotted against crude carotene content. Crude carotene in these samples ranged from 2.5 to 151 parts per million. Statistical analysis* of the data showed the relationship between true and crude carotene to be described by the equation

$$Y = 0.77x - 1.4$$

in which Y is true carotene and x is crude carotene when both are expressed as parts per million. The coefficient of determination was 0.9537 and the standard error of estimate 4.2.

BIBLIOGRAPHY

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