DETERMINATION OF VITAMIN A IN MARGARINE

BY

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Various difficulties are experienced in the quantitative determination of vitamin A. The Carr-Price reaction, in which the blue colour given with antimony trichloride is measured, is unreliable not only because of the transient nature of the colour but also because of the fact that other substances in addition to vitamin A give similar colours with the reagent. The measurement of the extinction in the ultra-violet at 325 mμ is far and away the most preferable, provided that no other substances are present which interfere with the absorption curve in the neighbourhood of this wavelength.

GRIDGEMAN and co-workers [1]) have considerably improved the determination of vitamin A in liver oil concentrates using this method by purifying the unsaponifiable part of such concentrates chromatographically (with Al_2O_3 as adsorbent) and by eliminating just those substances which interfere to a greater or lesser degree with the absorption curve of vitamin A in the ultra-violet.

However, for the determination of vitamin A in margarine, this method cannot be applied. Interfering substances, originating from the unsaponifiable part of the fats which are included in the margarine, cause an extremely strong distortion of the absorption curve of the vitamin A and they cannot be removed by chromatographic adsorption on Al_2O_3. It was therefore necessary in this case to carry out the determination using the older Carr-Price réaction, in which, however, widely varying results and often seemingly big "losses" occurred.

WILKIE and collaborators [2]) have suggested measuring the vitamin A in the chromatographed extract not at the maximum (325 mμ) but at 340 mμ, using an appropriate conversion factor. It was assumed that at this wavelength no irrelevant absorption would occur. Although this assumption has been found to be essentially correct, the method has the objection that the measurement takes place in a wavelength area in which the absorption curve rises very steeply. Slight deviations manifest themselves as big differences in the calculation of the vitamin A content so

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[1]) N. T. GRIDGEMAN, G. P. GIBSON and J. P. SAVAGE, Analyst 73, 662 (1948).

[2]) J. B. WILKIE, J. Assoc. Off. Agric. Chem. 30, 382 (1947); J. B. WILKIE and J. B. DE WITT, J. Assoc. Off. Agric. Chem. 32, 455 (1949).

that this method of determination must be considered as being in principle wrong.

In this laboratory, success has recently been attained in eliminating by a modified chromatography the substances causing the irrelevant absorption from the saponification extract, without loss of vitamin A. The measurement of $E^{1\%}_{1\,cm}$ 325 mμ and the use of the conversion factor 1900 gives the number of international units of vitamin A in the margarine. By this, the determination is adapted to the internationally established requirements of February 1950 [3]). The chromatography is carried out with two adsorbents connected in series. Al_2O_3 functions as the first adsorbent in the same way as it is used for concentrates. With this, substances which percolate more quickly that the vitamin A and which have a rather small absorption at 325 mμ are eliminated. The still contaminated vitamin A fraction which comes out of the column is then passed through a column of alkaline Al_2O_3 (10 % by weight of NaOH), the substances with irrelevant absorption being then completely retained (tocopherols and related compounds, as well as kitol). The form of the absorption curve of the vitamin A fractions from margarine thus obtained is pretty well identical with that of pure vitamin A and no correction is necessary. The procedure is carried out on a semi-micro scale with a total of only 200 I.U. vitamin A (so that only about 10 g margarine need be taken for the determination). The reproducibility of the method is very high (2—4 %) deviation), the recovery is better than 95 %, and the time taken is only about 2½ hours.

Full details of the procedure, reproducibility, and recovery will be published shortly elsewhere.

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[3]) World Health Org. Techn. Rep. Ser. 1950, 3.