agreed well with those obtained from calculations involving the iodine and thiocyanogen values; variations for linoleic acid were within 2 unit % while for oleic acid they were within 4 units %.

Discussion

The extraction of 14 varieties of sorghum grain under standardized conditions revealed variations between varieties of approximately 50% in the yield of wax and 20% in the yield of oil. These variations may have been influenced by soil fertility and climatic conditions as well as by genetic factors although all 14 varieties were grown during the same season in experimental plots under identical conditions.

Variations in climatic conditions might, however, influence the amounts of wax secreted in a given variety. Knaggs (9) noted such a relationship in the yield of carnauba wax; the carnauba palm reached its greatest productivity in periods of drought. The Western Blackhull kafir (1944 crop) reported in this paper contained 20% less wax than the sample from the 1943 crop reported previously (4). The annual rainfall during these two years was 29.7 and 16.1" respectively (10).

The slight variations in melting points indicate that the sorghum grain waxes probably do not differ greatly in composition. There was not enough wax obtained from the two samples of corn to determine an accurate melting point. Jamieson (11), however, reported a melting point of 81° C. for corn wax.

All of the waxes extracted with Skellysolve B were white in color. The pigment in sorghum grain can, if desired, be extracted with alcohol (12) or dilute alkali (13).

The characteristics of the oil extracted from the different varieties were similar to that of the Western Blackhull kafir previously reported (4). The percentage of nonsaponifiable matter, however, was approximately 0.5 units % larger.

Summary

Fourteen varieties of *Andropogon Sorghum* var. *vulgaris* were subjected to fractional solvent extraction. An average yield of 0.32% wax and 2.76% of oil was obtained.

The 14 sorghum grain oils varied from a light amber to green in color. They had an average refractive index of 1.4695 at 25° C. and contained 2.51% nonsaponifiable matter. The mixed fatty acids obtained from the oils had an average melting point of 28.9° C., a neutralization equivalent of 278.8, iodine value of 120.8, and thiocyanogen value of 81.5. The composition of the mixed fatty acids were calculated from the iodine and thiocyanogen values. The mixed fatty acids contained an average of 46.5% linoleic, 39.5% oleic, 7.8% palmitic, and 4.7% stearic acid.

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Report of the Vitamin Committee 1945-46

THERE are many scientific and commercial problems in the field of oil-soluble vitamins which need to be solved. The most urgent seemed to be the development of analytical methods which can be generally agreed upon. Methods for vitamin A and E were selected for first attention. Since vitamin A is better known scientifically and is probably more significant commercially at the present time, our attention was restricted to it during the last year.

A generally recognized need is for a vitamin A reference standard that can be useful for physical and chemical as well as biological tests. The present International Standard is a solution of carotene while the U. S. P. Standards have been a series of cod liver oils. Carotene, of course, is an entirely different substance from the vitamin A found in liver oils and in concentrates suitable for fortifying margarine, pharmaceuticals, etc. The cod liver oils are somewhat unstable and often give chemical reactions that are not typical of vitamin A. Our recommendation, given in detail in Table I, is that a solution of vitamin A acetate in vegetable oil be used as the reference standard.

There are at least two physicochemical methods that give fairly satisfactory determinations of vitamin A in certain high potency fish liver oils and concentrates. Unfortunately, they must be applied with considerable discrimination. The committee believes that it may be possible to work out a rigorous method which would cover most products of commercial interest. A proposed outline for such a method is shown in Table II. Much experimental work will have to be done to establish some of the points still in dispute.

Margarine is one of the most important commercial products containing vitamin A, and at the present

TABLE I

Vitamin A Reference Standard

Property	Physicochemical Requirements	Biological Requirements	Committee's Present Opinion
Form of Vitamin A	The substance $C_{20}H_{29}OH$ or ester	Same	Pure vitamin A acetate
Concentration	As high as possible to give little interference with physicochemical tests	As low as possible to simplify dilution with carrier oil	3,000 μg. of $C_{20}H_{29}OH$ per gram (about 10,000 U.S.P. units/gram)
Diluent	A non-volatile oil which will not interfere with physicochemical tests	A non-volatile oil which will not interfere with biological tests	Refined and deodorized cottonseed oil
Antioxidant	Must be oil soluble and not interfere with physicochemical tests	Tocopherol	1 to 3 mg. of tocopherol/g. (perhaps the natural content of cottonseed oil will be sufficient)
Container	Ampoule or capsule opaque to actinic light	Same	One-piece gelatin capsules

time it appears that it cannot be assayed by a method similar to that in Table II. The sub-committee on vitamin A of the National Association of Margarine Manufacturers is developing a special method for this product, and there is close cooperation between that committee and our own.

A few members of this committee are experienced in biological work, and we hope to make amendments to the present existing biological assay procedures. The most important problem here also seems to be the adoption of a suitable vitamin A reference standard.

TABLE II

Tentative Method for the Physicochemical Assay of Vitamin A

	Procedure	Detail
Basic Method	Determination of extinction coefficient at 326 to 328 mμ; calculate vitamin A content by direct proportion	Solvent to be isopropanol. Calibrate instrument with new vitamin A standard. Report in gravimetric units.
Necessary confirming data	(I) Determination of extinction coefficients at 300 and 350 mμ.	The ratios, E(300/E(328) and E(350)/E(328), to have values in prescribed ranges.
	(II) Determination of depth of color, at 620 mμ, of $SbCl_3$ reaction product; potency to be calculated by comparison with color produced by vitamin A standard	Potency to be determined by comparing reaction product of test solution with that of test solution fortified with standard. The color of the reaction product must fade at a prescribed rate. The potency by the color method must agree (within prescribed tolerance) with potency by U.V. method.
Purification Methods	(I) Saponification	Probably will be required
	(II) Distribution between solvents	Might be optional
	(III) Chromatography of unsaponifiable fraction	Might be optional
	(IV) Molecular distillation	Might be forbidden, except under conditions that would prevent pyrolysis of kitol into vitamin A

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Fractionation of Lard and Tallow By Systematic Crystallization†

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FRACTIONAL crystallization from solvents has been employed for more than a century in efforts to determine glyceride structure and composition of natural fats. The early work in this field, in addition to recent work up to 1939, has been discussed in considerable detail in a monograph by Hilditch (1) and more recently summarized by Brown (2). Practically all the early efforts were concerned primarily with the isolation of pure individual glycerides by fractional crystallization from solvents, usually at temperatures from room temperatures to 0°C. Much painstaking work showed that it was frequently possible by numerous crystallizations to isolate some of these glycerides in relatively pure form but gave little quantitative information concerning the glyceride composition of any fat.

About 1936, however, Hilditch and his associates employed crystallization technique from a somewhat different viewpoint. Instead of attempting to use it as a means of complete separation of fats into pure component glycerides, they applied it as a preliminary step in their chemical method of determining glyceride composition. The fat was separated by fractional crystallization from acetone at 0°C. into several fractions of less complexity than the original. Each fraction was then examined for its fatty acid composition, fully saturated glyceride content, and content of tri-C_{18} glycerides by determining the tristearin after partial or complete hydrogenation. With this analytical information available, they were able, with limitations, to deduce the approximate glyceride composition of the original fat on the basis of the assumption that the individual fatty acids are distributed as evenly as possible among the glycerol molecules. One of the limitations of this procedure is the inability to differentiate between the various types of mixed unsaturated glycerides, such as stearodioleins and stearooleolinoleins, and stearodilinoleins, which after complete hydrogenation are determined in the form of tristearin.

More recently (3, 4, 5), crystallization at much lower temperatures, —40°C. or lower, has been shown to effect considerable fractionation of the liquid glycerides. Indeed, Hilditch and Maddison (4) considered that sufficient separation had been achieved to justify

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