third of the saturated acids represented by the difference between the GS_3 content required by chance and that actually present with the unsaturated acids in the fat, without allowing the formation of any further GS_3.

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Sesame Oil. X.[1] The Stability of Vitamin A in Sesame Oil and in Margarine Stock Containing Sesame Oil

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CONSIDERABLE divergence of opinion exists in the literature regarding the stability of vitamin A in the presence of sesame oil. Conflicting views have been summarized by Budowski and Markley in an earlier paper in this series (2). Correspondence received in this laboratory not only indicates that confusion still exists but expresses a need for its clarification. Therefore it seemed desirable to include, in the investigations of the composition and properties of sesame oil, a study of the stability of vitamin A in the presence of this oil.

In many European countries the addition of small quantities of sesame oil to margarine is required by law to afford ready identification by means of the well known Villavecchia test. For this reason the stability of vitamin A used to fortify margarine, which contains a small quantity of sesame oil, is also of some practical interest. The stability of vitamin A added to margarine stock containing a small quantity of added sesame oil has been determined and compared to the stability of the vitamin in margarine stock containing no sesame oil. Results of stability studies on the sesame oil and margarine stock at two different temperatures are reported in this paper.

Description and Preparation of Samples

The lipide sample materials used in the investigations were: sesame oil, margarine stock, and a mixture of margarine stock and sesame oil.

The sesame oil was obtained from Colombian-grown seed by solvent extraction with commercial pentane. Following removal of the solvent at a low temperature, the crude oil was refined, bleached, and deodorized. The finished oil had the following characteristics:

Specific gravity	0.9219 25°/25°
Refractive index, $n_D^{40°}$	1.4673
Free fatty acid content	0.13%
Unsaponifiable matter	2.63%
Iodine value	116.2
Thiocyanogen value	78.0
Hydroxyl value	8.7
Peroxide value	0.0
Sesamoline content	0.35%
Sesamin content	1.06%
Color, Lovibond units	35 yellow and 0.47 red

Fresh margarine stock was obtained from a manufacturer of margarine oils. It was a mixture of 77.5% cottonseed oil and 22.5% soybean oil hydrogenated to margarine consistency.

The third sample was a mixture of the two samples just described in the proportion of 5% sesame oil to 95% margarine stock.

The sesame oil was divided into two portions. One was used as a control and the other was fortified with vitamin A concentrate.[4] Approximately 20 grams of the control portion was put into each of 14 1-oz. wide-mouth bottles fitted with plastic covers. They were stored in the dark, seven of them at a temperature of 32°F. and the other seven at 78°-80°F. Vitamin A was added to the second portion to give a fortified sample having a potency of 49.8 U.S.P. units per gram. This fortified sample was divided similarly into 20-gram lots and stored at the same two temperatures.

Portions of the margarine stock and the mixture of the margarine stock and sesame oil were fortified

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[1] For the preceding paper in this series see Analytical Chemistry, vol. 24, page 668, April 1952.

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[4] "Myvapack," natural ester form, 50,000 U.S.P. units per gram. (See footnote 5.)

in the same manner to contain 49.8 and 50.4 U.S.P. units of vitamin A, respectively. They were stored along with corresponding controls as described above.

Method of Analysis

Because an exact control for each sample to be evaluated was available, a direct ultraviolet absorption method for determining the vitamin A content of the fortified samples was used. This method is based on the measurement of the absorption of the vitamin A in solution, the concentration being proportional to the absorption at 325 mμ, the position of its maximum absorption (3, 5). Extraneous absorption at this wavelength was obtained by measurement of the control containing no added vitamin A. By using the value of this absorption as a correction factor, the necessity of applying the well known Morton-Stubbs correction (4) was eliminated. When the absorption of the controls is subtracted from that obtained for the corresponding fortified samples, the resulting value is that of the added vitamin A. This system of evaluation neglects only a possible minor absorption of oxidation products of vitamin A.

Obviously this method can be used only in those cases in which an exact control for each sample to be analyzed is available.

Experimental Procedure and Results

Samples of each of the fortified portions and the corresponding controls were analyzed for vitamin A after storage for 0, 1, 3, 7, 13, 26, and 42 weeks. The margarine stock and the mixtures of margarine stock and sesame oil were melted to facilitate thorough mixing. Approximately 2.5 grams of sample were weighed into a 50-ml. volumetric flask and dissolved in isopropanol of spectrophotometric quality. The exact weight of each sample was obtained to provide a basis for calculating the extinction coefficient. The solutions were filtered through Whatman No. 2 filter paper to insure clarity.

The instrument used for measurement of the filtrates was a Beckman DU spectrophotometer[5] equipped with a pair of matching 1-cm. quartz cells. One of the cells was filled with isopropanol and the instrument properly balanced at 325 mμ. The filtrate from each fortified sample and each control solution was in turn placed in the other cell and its optical density read at that wavelength. The extinction coefficients were calculated ($E = \frac{\text{O.D.}}{\text{g./l.} \times 1\ \text{cm.}}$). This procedure was followed in duplicate for each sample.

The average value of E for each pair of control samples was subtracted from each value of E for the corresponding pair of fortified samples. The amount of added vitamin A present in each of the fortified samples was then calculated by substituting in the following formula:

$$\text{U.S.P. units of vitamin A per gram} =$$
$$(E_1 - E) \times 5{,}700 \times \frac{1}{0.3}$$

where E_1 is the extinction coefficient of the fortified sample at 325 mμ; E the average extinction coefficient of the two unfortified controls at 325 mμ; 5,700 a factor for conversion from spectrophotometric to gravimetric units (1); and 0.3 a factor to convert grams to U.S.P. units. The results of the study are presented in Table I.

TABLE I

Percent Recovery of Vitamin A Added to Sesame Oil, Margarine Stock, and Mixture of Margarine Stock and Sesame Oil

Time of storage	Temperature of storage					
	Sesame oil		Margarine stock		Margarine stock plus sesame oil	
Weeks	32°F.	78-80°F.	32°F.	78-80°F.	32°F.	78-80°F.
0	100.8	100.8	104.0	104.0	101.8	101.8
1	103.2	101.8	104.8	108.4	103.0	101.6
3	103.8	101.4	106.8	105.6	109.8	109.3
7	105.0	100.6	99.4	111.8	107.5	104.0
13	105.0	98.8	103.4	103.8	107.7	107.9
26	99.6	82.3	111.8	98.2	100.4	97.8
42	95.0	61.8	105.4	94.0	95.8	95.4

Examination of the data in Table I leads to the conclusion that vitamin A is stable in sesame oil and in a mixture of 95% margarine stock and 5% sesame oil in samples stored at 32°F. for a period of 42 weeks. It is also stable in the margarine stock-sesame oil mixture stored at 78°-80°F. for the same period of time. The vitamin A in pure sesame oil stored at 78°-80°F. however began to show a definite trend toward instability between 13 and 26 weeks of storage, and after 42 weeks of storage the sesame oil samples measured showed a 40% loss of the vitamin A originally added.

Recoveries over 100% arise mainly from two factors. First is the experimental reproducibility of the ultraviolet absorption method. The Association of Vitamin Chemists (1) recognizes that duplicates should agree within ±5%, thus permitting recoveries of 105%. Secondly the factor recommended in the selected procedure to convert extinction coefficients to "biological units" is 1,900. Earlier a factor of 2,000 was used but subsequently found too high. Other studies have indicated that 1,600 would be a more nearly correct factor. A discussion of this factor is included in reference (1).

Summary

A study has been made of three groups of samples fortified with a vitamin A concentrate—sesame oil, margarine stock, and a mixture of 95% margarine stock and 5% sesame oil—to determine the stability of vitamin A in the presence of sesame oil. The samples were stored at two temperatures and their vitamin A content was measured periodically throughout a period of 42 weeks by an ultraviolet absorption method.

The results show no evidence of vitamin A instability in the three groups of samples after storage at 32°F. for 42 weeks, or in the margarine stock and margarine stock-sesame oil mixture after storage for the same period of time at 78°-80°F. However the pure sesame oil showed a significant change in vitamin A content at the end of 42 weeks of storage at 78°-80°F. The trend toward instability became evident after 13 to 26 weeks of storage.

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Solubility and Swelling of Metallic Ricinoleates in Solvents

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METALLIC ricinoleates belong to a class of materials known as metallic soaps, which in turn are commonly defined as the alkaline-earth and heavy metal salts of monobasic organic acids. Metallic soaps are characterized by a unique structure in that they combine in one molecule a *non-polar* organic radical with a *polar* carboxyl group having one hydrogen replaced by metal. It is the presence of these two dissimilar groups in a single molecule which accounts for the many interesting and commercially important applications for metallic soaps.

The metallic stearates (aluminum, zinc, calcium) and the metallic naphthenates (lead, cobalt, manganese, copper) are well known metallic soaps and account for about 70% of the annual United States metallic soap production of some 40,000,000 pounds (1949). Hundreds of other metallic soaps are theoretically possible, but relatively few have achieved the commercial importance of the metallic soaps noted above. In many cases this has been due a) to lack of knowledge concerning them and b) to the fact that they have not been commercially available.

Within the past year a group of soaps based on the fatty acids of castor oil, and known as the metallic ricinoleates, have been brought from relative obscurity to a stage where full scale production is now under way. Castor oil, itself, is differentiated from other fatty oils by the presence of a hydroxyl group on the 12th carbon atom of its principal fatty acid (ricinoleic acid). This feature leads to unusual properties, such as solubility of castor oil in alcohol, the only fatty oil to be so soluble. In turn it was conjectured that the presence of this hydroxyl group might also confer unique solubility properties to those metallic soaps derived from castor oil.

It is the purpose of this paper to report on five of these soaps and specifically on their solubility and swelling characteristics. It is felt that this information will be of distinct value in developing useful applications for these relatively new products.

Three of these metallic ricinoleate soaps (or salts), based on the fatty acids of castor oil, are being supplied to the trade as fine white powders. In appearance they are quite similar to their metallic stearate counterparts. A fourth metallic ricinoleate, magnesium ricinoleate, is supplied in the form of coarse white granules which tend to fuse together on standing. The fifth metallic ricinoleate, aluminum ricinoleate, is supplied as a taffy-like product. Metallic stearates corresponding to these five metallic ricinoleates are also included as reference materials in developing this solubility information.

The literature has little to say about the solubility of metallic ricinoleates. For example, Doss (1) in his recent excellent and comprehensive compendium of data on fatty acid salts was able to locate and report only the briefest information on metallic ricinoleate solubility as drawn from six references out of a total of 612 references on metallic soaps in general. Hence it was decided to determine in some detail the solubility characteristics of aluminum, barium, calcium, magnesium, and zinc ricinoleates. Only factual data is reported, and no attempt has been made to theorize or otherwise interpret the results obtained.

In planning the study on solubility, it was decided to work first with only two concentrations (25% and 50%) of the five metallic ricinoleates in selected solvents. From this screening evaluation more detailed study could be made as indicated. Parallel solubility tests were also run on the five corresponding metallic stearates to give a comparison reference system.

The 25% and 50% solution concentrations were made up as follows in some 25 solvents:

Procedure for Preparing 25% Concentration of Metallic Soap

a) 25 g. of soap were placed in a tared 250-ml. Erlenmeyer flask.
b) 75 g. of solvent were added.
c) The contents of the flask were refluxed on a steam bath for three hours.
d) The flask was then removed from the steam bath and immediately stirred to insure a uniform composition.
e) Before allowing the mix to cool to room temperature, a representative sample was poured into a 50 ml. test tube.
f) The filled test tube was stoppered and placed in a rack for observation after aging at room temperature for 24 hours and 30 days.

Procedure for Preparing 50% Concentration of Metallic Soap

a) 50 g. of soap were placed in a tared 250-ml. beaker.
b) 50 g. of solvent were added, and the mix was stirred to insure a uniformity of composition.
c) This mix was then passed through a three-roll laboratory paint mill three or four times to achieve a uniform dispersion of soap in the solvent
d) The dispersed product obtained by the milling procedure was returned to the 250-ml. beaker and the solvent loss sustained during milling determined.
e) Any required makeup of solvent was added, and the mix was given a final pass through the mill.
f) A 50-ml. test tube was filled with the final dispersion and stoppered.
g) The filled test tube was then placed in a rack and observed after aging at room temperature for 24 hours and 30 days.

A mechanical dispersion method was used for obtaining the 50% soap concentrations since it was believed some difficulty might be experienced in