The above have been established as preferred conditions although preliminary work was done with 70% perchloric acid, which causes more rapid isomerization and introduces more reversion. With this strength of acid a 90-second interval with a factor of 1.15 can be used and was the method of analysis shown in Table I.

The precision and accuracy seem reasonable in the light of the limited work reported. The analytical method, which combines Martin's isomerization with the periodic acid oxidation procedure of Handschumaker and Linteris (11), consists of the following steps:

1. Dissolve a weighed sample (which contains approximately 100 mg. total monoester, glycerol-free by water washing in ether solution) in 15 ml. of 2:1 acetic acid:chloroform in a 250-ml. iodine flask.
2. Add 0.045 ml. of 56% $HClO_4$, shake for 60 seconds, and let stand for 9 minutes.
3. Add the 25 ml. periodic acid reagent and continue with the analysis as described by Handschumaker and Linteris (11).
4. Calculate the total monoglyceride content by multiplying the result of that analysis by 1.15.

Composition of Monoglycerides

Isomerization analyses indicated about 5 to 8% of 2-monoglyceride in distilled products; mono-diglycerides contained about half as much total monoglycerides with about 5% of this also being 2-isomer. For example, one product at 37.8% 1-monoglyceride analyzed for 39.6% total monoglyceride. Countercurrent distributions and chemical analyses were confirmatory. For example, hydroxyl values of distilled monoglycerides ranged from 316 to 322 in material where 322 was theoretical for 100% monoglyceride.

The crystallization residues contained, by a variety of techniques, about 25 to 30% 2-monoglyceride, which corresponded to 4 to 5% 2-monoglyceride in the distilled product. This figure is, of course, a minimum since the crystallization was not necessarily a quantitative separation.

As a result, it is believed that all commercial monoglyceride products contain 2-monoglycerides. The amount is probably 5 to 8% of the total monoglyceride content.

Summary

1. Commercial monoglycerides and mono-diglycerides contain 2-isomers. The amount is in the range of 5 to 8% of the total monoglyceride content.

2. 2-Mono-olein and 2-monostearin have been isolated from the reaction products of glycerin with oleic and with stearic acids.

3. At least for cake baking utility, 2-monoglycerides appear to be equivalent to 1-monoglycerides.

4. An analysis for total monoglyceride content is proposed. It incorporates perchloric acid isomerization directly into the usual periodic acid analysis.

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Accelerated Stability Test for Vitamin A in Oils and Fats by Means of Surface-Enlarging at Room Temperature

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MANY ACCELERATED METHODS have been proposed and used for the study of relative stability to oxidation of fats, oils, and oil-soluble vitamins. Among these are the Active Oxygen Method (frequently referred to as the "Swift" method) (1, 2, 4, 7), the Schaal oven test (2, 6, 7, 12), and the Barcroft-Warburg manometric method (8, 10, 14). All of these methods have the weakness of being conducted at temperatures higher than those of ordinary storage, thus raising a question as to the mechanism of the reactions, which would be involved under these conditions, as compared with those in more nearly "normal" circumstances (15). For example, it has been found that N.D.G.A. has a diminished effectiveness at relatively high temperatures (3, 9, and author's own data). Similar observations have been made with respect to isobutylgallate (11). The use of metallic catalysts such as copper (12) for accelerating oxidation also raises a question as to the reactions involved.

Dubouloz (5) has accelerated oxidation by expanding the surfaces through the medium of spreading vitamin A oil on filter paper. These papers are then suspended in a closed container at a constant temperature between 90° and 100°C. Taufel (15) also developed a method employing surface-expansion, using chromatographic paper in diffuse daylight at room temperature; but the use of diffuse daylight as a catalyst presents a condition not often found in regular storage conditions of vitamin A substances.

The present paper proposes an accelerated test for the relative stability of vitamin A-bearing oils which attempts to stay as closely as possible to ordinary storage conditions and still offer the merits of an

TABLE I

Various Oils, Examined in the Glass Bead Test

Trial number	Oil	Initial content, I.U. vitamin A/g.	Peroxide value, milli aeq./kg.	Acidity, mg. KOH/g.	H(alfway) V(alue) 50% loss, vit. A (days)
	Synthetical product, brand A	*approximately*			
1	Vitamin A acetate	1,000,000	4	1.7	>7[a]
2	Duplicate of no. 1	1,000,000	4	1.7	7-8
3	Vitamin A palmitate	1,300,000	12	1.3	15
4	Duplicate of No. 3	1,300,000	12	1.3	15
	Synthetical product, brand B				
5	Vitamin A acetate	1,100,000	43	2.1	5
6	Duplicate of no. 5 (after 8 months)	1,100,000	47	2.2	5-6
7	Vitamin A palmitate	1,100,000	31	2.3	13
8	No. 7 + mixture of antioxidants	1,100,000	5	4.6	24-25
	Vitamin A acetate from natural oils after saponification, extraction and acetylation				
9	Vitamin A acetate	750,000	9	1.4	2-3
10	Duplicate of no. 9 (after 1.5 months)	750,000	9	1.4	2
11	No. 9 + 0.1% N.D.G.A.	750,000	4	1.1	4-5
12	No. 9 + 0.1% N.D.G.A. + 0.05% c.a.	750,000	2	1.5	4-5
	Molecular distillation products of natural esters				
13	Vitamin A molecular distillation	1,500,000	10	4.2	3-4
14	Duplicate of no. 13	1,500,000	10	4.2	3-4
15	Oil of low stability[b]	900,000	17	....	0-1
16	No. 15 + 0.3% N.D.G.A. + 0.05% c.a.	900,000	3	....	4
17	Vitamin A molecular distillation	100,000	7	0.6	5-6
18	No. 17 + 0.3% N.D.G.A. + 0.1% c.a.	100,000	5	1.6	>15[a]
19	Vitamin A molecular distillation	600,000	23	0.8	1-2
20	No. 19 + 0.3% N.D.G.A. + 0.1% c.a.	600,000	22	0.9	>12[a]
21	No. 19 diluted with paraffin oil	125,000	2	0.8	3-4
	Whale-liver-oil concentrates				
22	South African	100,000	17	1.7	8-9
23	Japanese	200,000	6	1.0	3-4
	Shark liver oils				
24	Brand C	2,300	20	4.0	3-4
25	Duplicate of no. 24	2,300	20	4.0	3-4
26	Brand D	260	180	4.7	0
27	Duplicate of no. 26	260	....	....	1
28	Brand E	2,400	7	2.3	11-12
29	Duplicate of no. 28	2,400	7	2.3	13-14

[a] > Means: all 10 dishes tested, percentage still over 50.
[b] This oil has proved in practice to be of low stability.

accelerated method. That is, the temperature used is 20°C. in a thermostatically controlled oven, and light is excluded. The accelerated oxidation is obtained by the use of a vast surface-expansion resulting from the spreading of the oil over a neutral carrier, *i.e.*, glass beads. The rate of progress of the oxidation (and attendant deterioration of the vitamin A) is followed by making direct determinations of the vitamin A content at suitable periods of time. The Carr-Price method for vitamin A is used. Supplementary data on peroxide and acid values are included.

Preparation of the Glass Beads

Glass beads with a diameter of about 2.2 mm. were cleaned, using alkaline alcohol, distilled water, concentrated hydrochloric acid, and distilled water successively. (On beads selected at random, diameter measurements were found to vary from 1.8 mm. to 2.25 mm.) Used beads were first degreased with chloroform and then cleaned as described above.

Procedure of the Test

The oil is weighed into a Petri-dish (about 0.0625 g.) then dissolved in a few ml. of pure, moisture-free petroleum ether (40-60°C.). Cleaned glass beads are then added in sufficient number to cover the bottom levelly (about 4 g.). The petroleum ether is then evaporated at room temperature in the dark and finally the dish, loosely covered, is placed inside a 20°C. oven. A number of dishes are so prepared and stored for each sample. At intervals a dish is removed from the oven, and the contents are analyzed for vitamin A. These results are used to determine the time in days for the vitamin A content to decrease to 50% of the original. In determining the vitamin A, it is convenient to use either chloroform or thiophene-free benzene and to rinse the sample into an appropriate-sized flask so that the concentration will be suitable for the antimony trichloride reaction. Determinations thus made directly are sufficiently accurate for comparative purposes.

Application to Fish Liver Oils with a Low Vitamin A Content

Because these oils require saponification prior to the determination of vitamin A, the use of conical flasks instead of Petri-dishes simplifies the procedure. These flasks should have a flat bottom of about 4.2 cm. diameter and should be about 8 cm. high. The oil is weighed into the flasks as in the Petri-dishes, petroleum ether and beads are added, etc. The petroleum ether is evaporated, using a stream of carbon dioxide followed by air for about 1 min., and the loosely covered flasks are placed in the 20° oven. When a flask is removed for vitamin A determination, 5 ml. of alcohol are added, followed by 0.5 ml. of 60% KOH. The contents are then saponified for 5 min. in a water bath, and the determination is carried out in the usual way.

Using the above described procedures, approximately 100 vitamin A containing oils were tested, using synthetic as well as the natural products together with molecular distillates with and without added antioxidants. Some of the results obtained are shown below in Table I.

Surface Expansion

The approximate increase in surface may be calculated as follows. Four grams of beads (average count 390) have a total surface of about 4925 mm.^{2} (assuming average diameter as 2.0 mm.). The surface of 0.0625 g. of oil (assuming a spherical shape)

is about 81 mm.[2] Hence the expansion is 4925/81, or about 60-fold. Obviously this calculation is an approximation which ignores any spreading of the oil over the sides and bottom of the dish. The expansion would be still greater if compared with the oil in a container in which the exposed surface is less than that of a sphere.

It is noteworthy that doubling the quantity of beads will yield approximately the same stability figures on the vitamin A as are found using the smaller amount of beads. Practically, this means that the beads need not be weighed accurately each time and that measuring the quantity in a graduated cylinder is a sufficiently accurate procedure. The explanation of this seemingly anomalous fact is as follows.

On examining the oil-covered beads under a microscope, it was observed that a major portion of the oil was accumulated *between* the beads as saddle-shaped, parabolic hyperbolic forms and that only a minor portion of the oil was actually in the form of a film over the beads themselves. In fact, the amount of oil on the beads was so slight that the use of ultraviolet light to produce fluorescence failed to disclose the presence of an oil film. When Sudan III was added to darken the oil however, a thin film could be observed on the beads.

The following theory is therefore proposed. The oil forming a film on the beads is very small in comparison with the mass of oil lying between and among the beads, but it has an enormously expanded surface. The oil between the beads, while composing the major mass of material, possesses only a very small surface with relation to its volume. It follows that if the film surface available is increased by using more beads, the surface is increased, but the mass of oil comprising this increase is very small with respect to the remaining oil. Hence it has only a small influence on the vitamin A found. With the quantity of beads greatly increased, say 15-fold (or the amount of oil drastically reduced), there remains only a very little oil between the beads and the ratio of surface to volume is greatly increased. In addition, the total amount of oil exposed to the air is relatively large. It was actually observed that deterioration was considerably increased in tests employing only one-fifteenth the usual quantity of oil. For example, an oil (570,000 I.U./g.) with a half-way value of about 30 days in the regular test was found to have a half-way value of 3 to 4 days when the amount of oil was reduced to one-fifteenth. In another experiment an oil (520,000 I.U./g.) yielded results of 27 days and 3 to 4 days, respectively.

Comparison with the Active Oxygen Method ("Swift" Test)

A number of oil samples were submitted to both the bead test and the A.O.M. test, the latter at 95°C. The experiments were performed by different people in this laboratory as separate tests. For the A.O.M. test the oils were diluted with paraffin oil or squalene to a potency of about 10,000 I.U./g. This dilution was made in accordance with the work of Debodard (4), who showed the influence of potency level on stability.

In general, the relative stability values contained in the A.O.M. tests were higher as the dilutions increased. Debodard states that the oils should be tested after dilution. Hence the values from the bead-tests on undiluted oils were compared with values obtained by the A.O.M. on oils diluted to a potency of 10,000 I.U./g. Table II presents the data obtained, showing the values in days for the bead tests, and in hours for the A.O.M. tests.

Graph 1 shows stability tests on various samples of vitamin A oils as determined by the bead test, compared with data obtained by the A.O.M. (The half-way values refer to 50% destruction of vitamin A.)

Discussion

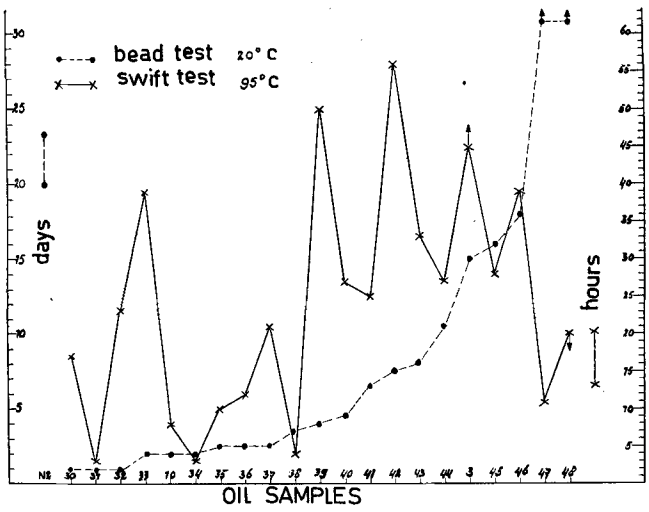
It seems clear that there are striking differences in the data obtained by both tests, particularly in Samples 34 and 47 (these samples were composed of crystalline vitamin A acetate dissolved in squalene, and contained respectively 100,000 I.U./g. and 10,000 I.U./g.). The half-way values were, in the bead test, 2 days and 34 days; in the A.O.M. tests, 3 hrs. and 11 hrs.

Certain of the evidence seems to favor the bead test as a means of showing differences in stability. For example, Sample 48 was an oil that was known to be of good stability, good enough, in fact, to be used as a standard for calibrating spectrophotometers.

TABLE II

Values of the Glass Bead Test as Compared with the Swift Test

No.	Sample of oil	I. U. vit. A/g.	50% vitamin A remaining after		Peroxide value mg. aeq./kg.	Acidity, mg. KOH/g.
			Bead test, days	Swift test, hours		
30	Synthetical product, brand B, vitamin A acetate	550,000	1	17	14	1.0
31	Molecular distillation of natural esters	110,000	1	3	19	0.3
32	Synthetical product, brand B, vitamin A palmitate	1,230,000	1	23	3	1.1
33	Synthetical product, brand B, vitamin A palmitate	1,120,000	2	39	5	1.4
10	Vitamin A acetate from natural oil, after saponification, extraction, and acetylation	750,000	2	8	12	1.4
34	Crystalline vitamin A acetate, brand A, in squalene	100,000	2	3	1	0.7
35	Vitamin A acetate from natural oil, after saponification, extraction, and acetylation	1,430,000	2-3	10	7	3.9
36	Vitamin A acetate from natural oil, after saponification, extraction, and acetylation	1,380,000	2-3	12	9	2.1
37	Synthetical product, brand A, Vitamin A palmitate	1,900,000	2-3	21	21	1.8
38	Molecular distillation of natural esters	1,690,000	3-4	4	27	3.2
39	Synthetical product, brand F, vitamin A palmitate	1,080,000	4	50	13	1.1
40	Natural ester concentrate, brand F	250,700	4-5	27	17	0.9
41	Synthetical product, brand A, vitamin A palmitate	1,200,000	6-7	25	17	1.4
42	Synthetical product, brand F, vitamin A acetate	870,000	7-8	56	17	1.5
43	Synthetical product, brand B, vitamin A palmitate	1,260,000	8	33	12	1.2
44	Synthetical product, brand G, vitamin A palmitate	1,100,000	10-11	27	22	2.2
3	Synthetical product, brand A, vitamin A palmitate	1,260,000	15	>45	7	1.2
45	No. 44, diluted with soybean oil	515,000	16	28	18	3.4
46	Synthetical product, brand B + vitamin A, acetate + antioxidants	1,130,000	18	39	18	2.4
47	Crystalline vitamin A acetate, brand A in squalene	10,000	>34	11	2	0.7
48	Natural vitamin A ester, brand H	54,000	40	<20	3	2.7



GRAPH 1

The bead test value was 40 days compared with 20 hrs. by the A.O.M. If the oil had not been diluted the A.O.M. value would have been still lower. Also, comparing Sample 44 with Sample 45, the influence of dilution is quite evident in the bead test (a rise from 10-11 to 16 days) while the A.O.M. showed virtually no difference (27 hrs. to 28 hrs.). Actually this comparison of the two methods was not sufficiently comprehensive to justify firm conclusions.

Conclusion

It is believed that the above described experiments disclose a method which merits further investigation and application as a means of determining the stability of vitamin A in oils and fats. The values found are reproducible within a deviation of about one day (at large half-way values the absolute variation would probably increase). The times between testing and retesting varied from a few days to about 8 months (sample stored under CO_2 or nitrogen at 5°C.).

An outstanding aspect of the data presented shows the improved stability due to the various antioxidants, for example, Sample 7 compared with Sample 8; Sample 15 compared with Sample 16, etc. Another interesting disclosure is the influence of dilution on stability.

It is important to point out that the value of this (or of any other) accelerated method may not be judged with confidence until the method has been compared with the results of tests under ordinary storage conditions. These storage experiments are in fact under way at the present time but are not yet completed.

It should be pointed out also that the tests described may be still further accelerated by using smaller quantities of oil than those described.

The use of an accelerated test for vitamin A stability, based on surface-expansion, is in better accordance with practical conditions than are other tests because of the practical application of vitamin A oils in dry-feed mixtures, tablets, etc.

Summary

A new, simple, and rapid method is described for evaluating the stability of vitamin A in oils.

The acceleration is obtained at room temperature (20°C.) by spreading the oil over a neutral carrier (glass beads), thus obtaining greatly expanded surface areas, and consequent increased exposure to air.

Comparative tests were conducted involving the Active Oxygen Method ("Swift" test) and, while the results were inconclusive, striking deviations in results were noted.

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The Suppression of Soil Redeposition[1]

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IT IS DESIRABLE to consider the redeposition of soil as a separate entity in the total washing process. Soil redeposition is caused primarily by presence of calcium and magnesium cations found in natural waters and to a lesser degree by the relatively high concentration of sodium ions introduced with the washing composition.

Examining the deposition of carbon from aquadag suspensions and the redeposition of soil in the washing process, P. T. Vitale (1) has recently drawn attention to the large effect produced by electrolytes and particularly to the presence of divalent cations.

In the present study the deposition effects from Aquadag[2] suspensions are examined as quantitatively as possible in order to evaluate the suppression of the

[1] Presented at the fall meeting, American Oil Chemists' Society, Minneapolis, Minn., Oct. 11-13, 1954.

[2] Acheson Colloids Corporation, Port Huron, Mich.