as a product of the oxidation of unsaturated fatty acid esters.

Discussion

The yellowing of drying oil films seems to involve two distinct steps, the formation of colorless precursors by an oxidative mechanism, followed by a further reaction of the precursors to give the yellow compounds. Only subtle differences were found between the yellow compounds and their precursors. The yellowing probably involves a reaction of active centers in the precursor molecules in some type of condensation. Two observations support this view: a) yellowing can be induced by heating aged, nonyellowed films *in vacuo* (nonoxidative process), and b) low-molecular-weight aldehydes containing an active methylene group are effective inhibitors of yellowing.

No specific physical or chemical property could be related quantitatively to the yellow compounds or their precursors. Two problems were responsible: the inhomogeneity of the colored compounds and the difficulty of concentrating them free from noncolored substances. If the yellow compounds are highly colored, only a few tenths or hundredths of 1% may be all that is necessary to produce the color normally observed in aged films. The strong hydroxyl and carbonyl absorption that characterizes the infrared spectra of aged films of drying oils is not associated directly with yellowing. This does not preclude the possibility that the yellow compounds and their precursors are carbonyl compounds. In fact, since they are soluble in dilute alkali, one might suspect that they are related to hydroxy quinones. However no quinonoid structures could be detected by ultraviolet spectral analysis. This contrasts sharply with the results of McAdie and Nicholls (8), who found that a strong absorption band at 250 mμ was formed on the aging of films of methyl linoleate and linolenate. This observation formed the basis of their theory that benzoquinone structures which have an absorption band at 245 mμ are produced in oxidized films, and led to the suggestion that quinonoid structures are responsible for the color of yellowed films. Failure to observe any strong absorption at 250 mμ, since absorption at this wavelength occurred about equally in films of linoleate and linolenate esters (8), indicates that yellowing in film must be largely explained in some other way.

The observed inhibition of yellowing by chemical additives is significant because it portends the possible development of inhibitors suitable for practical application. The function of the inhibitor seems to be different from that of an antioxidant in retarding autoxidation. In yellowing, the function of the inhibitor appears to be to substitute for some reactant in a way that will yield nonyellowed adducts with the colorless precursors. Thus a sufficient amount of inhibitor is required to form adducts with all of the precursor molecules. Nevertheless if, as suspected, the major yellowing compounds are formed in only minute amounts, only small amounts of inhibitor should be required to prevent yellowing, provided that sufficiently reactive inhibitors can be found.

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An Accelerated Test of the Yellowing Tendency of Drying Oils[1]

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An accelerated test for the determination of the yellowing tendency of oils is described.

The test is demonstrated on purified esters as well as on a number of common oils used as vehicles in paints and varnishes.

[1]A report of work done under contract with the U.S. Department of Agriculture and authorized by the Research and Marketing Act; it was supervised by the Northern Utilization Research and Development Division of the Agricultural Research Service, Peoria, Ill.; it was supported also by The Hormel Foundation.

BECAUSE the yellowing of oils is influenced by many factors (1,2) and the colored bodies formed in the reaction have not been well characterized in terms of chemical and physical properties, it is not possible to predict the susceptibility of paint and varnish vehicles to this phenomenon on the basis of chemical examination of the vehicle.

Johnston and Fitzgerald (1) studied the effect of a number of factors on yellowing and gloss in laboratory tests, using white vitrolite glass panels as the substrate for the films and chromaticity measurements for measuring the degree of yellowing. Although the results were significant, there were gaps and discrepancies which were attributed by them to deficiencies in their experimental methods.

During the course of our studies on the yellowing of oils (2) it became highly desirable to have a simple quantitative test for yellowing which would measure the yellowing tendency of paint and varnish vehicles and for speed and convenience could be carried out in the laboratory. This paper describes such a test.

Materials

Highly-purified methyl linoleate, linolenate, and docosahexaenoate were obtained from the Hormel Foundation. The methyl linoleate was prepared from safflower seed oil and was more than 99% pure. The methyl linolenate was prepared from linseed oil by the bromination and debromination procedure and was also more than 99% pure. Methyl docosahexaenoate was prepared from tuna oil and was about 90% pure. A methyl linolenate concentrate was prepared by urea fractionation of the methyl esters of linseed oil fatty acids and contained 86% linolenate as determined by alkali-isomerization analysis.

Methyl esters of soybean oil and safflower seed oil fatty acids were obtained from J.C. Cowan, Oil Seed Crops Laboratory, Northern Utilization Research and Development Division, U.S. Department of Agriculture. These products were colorless and free of oxidation products.

Methyl esters of linseed oil fatty acids were prepared by interesterification of linseed oil with methanol, using sodium methoxide as the catalyst. The crude esters were distilled to give a final product which was colorless and had an iodine value of 185.0.

Menhaden and herring oils of good quality were obtained from the United States Department of Interior, Fish and Wildlife Service, Fishery Technological Laboratory, Seattle, Wash.

The other oils and alkyds used in this study were obtained from commercial sources.

Procedure

Preparation of Film. A 40 x 55-mm. rectangle of Whatman No. 1 filter paper is placed on a small aluminum wire support (Figure 1) on the pan of an analytical balance and weighed. Then about 50 mg. of sample are placed drop by drop in the center of the paper.

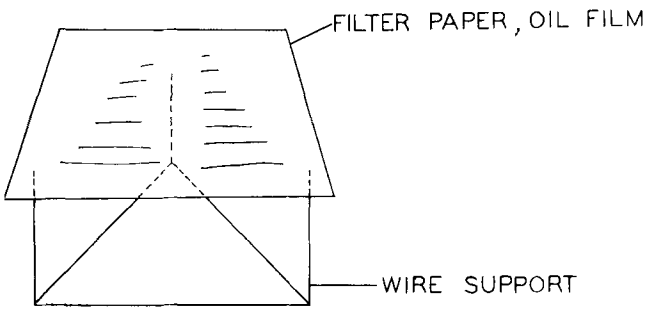

FIG. 1. Filter paper film (40 x 55 mm.) on aluminum wire support.

As soon as the sample is placed on the filter paper, the unit is placed in a desiccator where it is kept under nitrogen at reduced pressure for about 1 hr. The purpose of this is to give the sample time to diffuse over the surface of the paper.

The "filter paper film" is then removed from the aluminum support with a pair of forceps and placed in a 9-cm.-in-diameter Petri dish on two small glass rods to permit free contact of air on both sides of the filter paper. The cover of the Petri dish is held ajar by three small pieces of nichrome wire bent over the side of the dish. The covered Petri dish is placed in a forced draft oven at 50°C., and at appropriate intervals the filter paper film is removed and the amount of yellowing is determined in a Beckman Model DU spectrophotometer, equipped with a reflectance accessory. The short intervals in which the film is outside of the oven for measurements do not interfere with the progress of the test. Thus a time-*vs.*-yellowing curve can be obtained on a single film.

Measurement of Yellowing. A 40x55 mm. rectangle of filter paper is used for the film because this size fits the slot in the reflectance housing of the Beckman spectrophotometer. The amount of yellowing is determined by the difference in the absorbance measurements (expressed as △ optical density) at 520 mμ and 650 mμ, using a clean white filter paper as the reference standard. When this value is plotted against time, the general relationship shown in Figure 2 is obtained. The onset of yellowing is determined as the point where the curve separates from the base line as demonstrated in the insert in Figure 2(A').

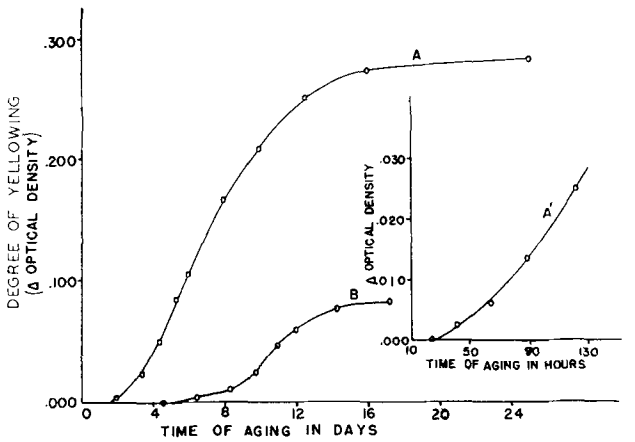

FIG. 2. Accelerated yellowing test for A, methyl linolenate, and B, methyl linoleate at 50°C.; A' shows the initial stages of the changes in the optical density curve for methyl linolenate.

Results and Discussion

Tests on a number of common oils and related derivatives are reported in Table I. In general, the onset of yellowing of these oils is related to the types and proportions of the polyunsaturated fatty acids and fall in the general order of their known yellowing tendencies. Hence the test appears to give a valid measure of the relative tendencies of oils to yellow. In addition to fatty acid composition, the age and previous treatment of oils also have an effect on their

TABLE I
Accelerated Yellowing Tests on Various Oils at 50°C.

Sample	Iodine value (Wijs)	Onset of yellowing (hrs.)
Menhaden oil	176	39
Herring oil	115	54
Raw linseed oil	180	105
Safflower seed oil	142	113
Alkali-refined soybean oil	129	142
Cottonseed oil	115	220
Methyl linoleate	173	70
Methyl linolenate	259.4	30
Methyl docosahexaenoate	432.0	6
Linseed long-oil alkyd	.....	49
Soybean long-oil alkyd	.....	79
Tall oil long-oil alkyd	.....	158
Safflower short-oil alkyd	.....	195
Soybean short-oil alkyd	.....	168

susceptibility to yellowing, and these are also reflected by the test (2).

Precision and reproducibility of the test were indicated by the results on methyl linolenate, which did not vary by more than 30 min. ($\pm<2\%$) determined at various intervals over a six-month period.

Determination of the amount of yellowing by the changes in chromaticity, as employed by Johnston and Fitzgerald (1), offered no advantage over the present method as a measure of the yellowing tendency. Measurements of chromaticity changes are impractical for an accelerated test such as described here. The change in chromaticity of films of methyl linoleate and methyl linolenate aged for various periods of time under the conditions of our test are shown in Figure 3. These results show that the tendency to yellow must be related arbitrarily to the slope of the curves for quantitation.

Figure 4 shows the spectra of films of a concentrate of methyl linolenate aged for various periods of time. The onset of yellowing detected visually corresponded closely to the first detectable decrease in reflectance of 520 mμ. This, and the observation that little decrease in reflectance occurred beyond about 620 mμ even in darkly-colored films except that due to a decrease in brightness, led to the selection of the conditions of this method for measuring yellowing. In addition to being fast and simple, the method has the advantage that irrevelant differences due to gloss and brightness are cancelled out.

Filter paper was selected as the substrate for the films because it proved to be the best surface for the preparation of uniform films. Surfaces that are normally used in practice, such as wood. are not chemically neutral, and glass, which is often used, is subject to the difficulties of irregular distribution and "crawling" which make accurate measurements of the yellowing tendency impossible.

The amount of sample may be varied between about 30 and 80 mg. for this size of paper (40 x 55 mm.) with very little variation of the onset time (Figure 5). This holds for concentrations of methyl linolenate in methyl myristate as low as 5%, which is well below the concentration of the polyunsaturated fatty acids in common dry oils. Samples less than 20 mg. give erratic results because this amount of oil does not completely cover the paper. On the other hand, with samples of more than 100 mg., the paper leaves significant amounts of oil on everything it touches.

In some cases in which completely different types of vehicles are being compared, it may be desirable to measure the extent of yellowing as determined from the plateau of the curve (*cf.* Figures 2 and 5).

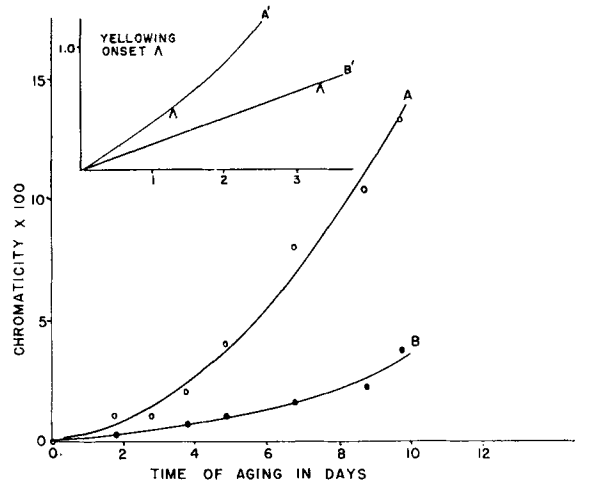

FIG. 3. Changes in chromaticity of films of methyl linolenate (A and A') and methyl linoleate (B and B') during aging in accelerated yellowing tests at 50°C.

Chromaticity =

$$\left[\left(\frac{X}{X+Y+Z}-X_1\right)^2+\left(\frac{Y}{X+Y+Z}-Y_1\right)^2\right]^{1/2}$$

where X, Y, and Z are tristimulus values calculated from the B and L Trichromatic coefficient computing form for Illuminant C and $X_1 = 0.3101$ and $Y_1 = 0.3163$ for the trichromatic coefficients for Illuminant C.

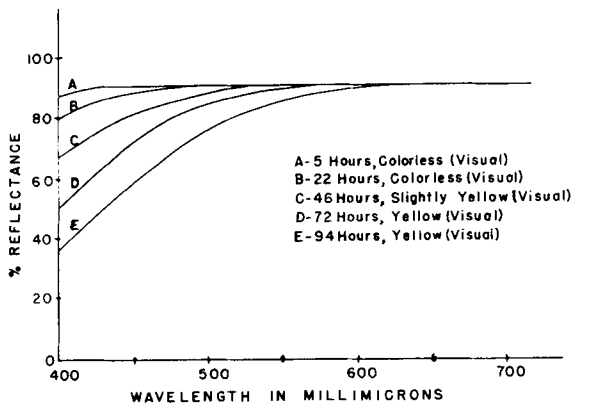

FIG. 4. Reflectance spectra of films of distilled methyl esters of linseed oil aged for various periods of time.

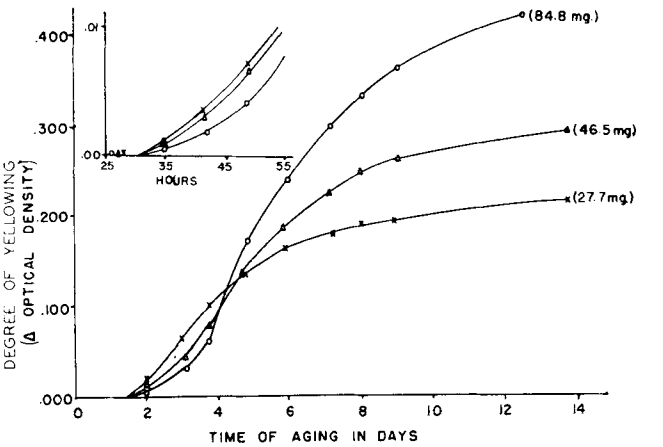

FIG. 5. Effect of sample size on the yellowing of methyl linolenate in accelerated test at 50°C.

However the extent of yellowing is greatly influenced by the amount of sample (film thickness). Thus the amount of sample and size of the paper must be carefully controlled for reproducible results. Further, although the yellowing curve reaches a relative plateau (Figures 2 and 5), it continues to increase slowly. This means that an arbitrary point on the curve must be selected, and this may lead to errors especially when comparing compounds with widely different rates of yellowing. However, for oils with similar yellowing characteristics, one can obtain good reproducibility, and a linear relationship between the concentration of yellowing precursors and extent of yellowing (Figure 6) when the above precautions are taken. Figure 6 shows the relative extents of the yellowing of samples of linseed oil methyl esters diluted with various amounts of methyl myristate.

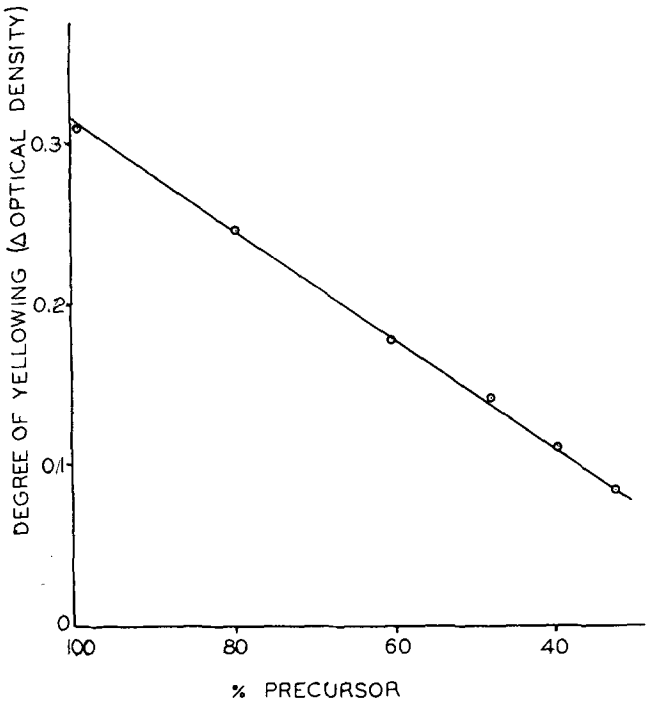

FIG. 6. Relationship between extent of yellowing and concentration of linseed oil methyl esters (yellowing precursor) in methyl myristate. Films consisted of 50 mg. of sample spread on 40 x 55 mm. rectangle of filter paper and were aged for 475 hrs. at 50°C. in the dark.

The onset time of yellowing generally will provide the same information on yellowing tendency as the extent of yellowing and has the advantage of being essentially insensitive to film thickness (Figure 3), is much faster, and can be determined with greater precision. It also forms a linear relationship with the log of the concentration of the precursors. This is

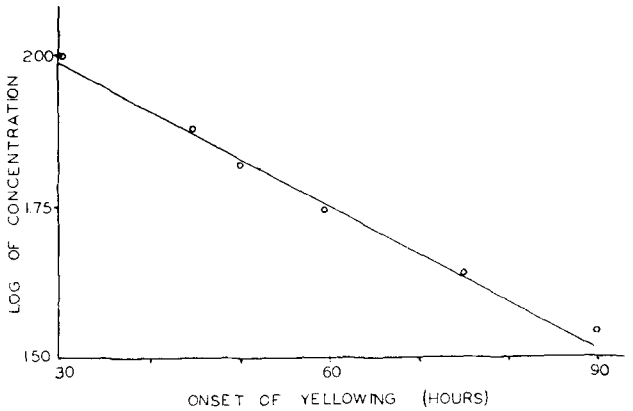

FIG. 7. Relationship between onset yellowing time and concentration of methyl linolenate (yellowing precursor) in methyl myristate. Films consisted of 50 mg. of sample spread on a 40 x 55 mm. rectangle of filter paper and were aged at 50°C. in the dark.

demonstrated in Figure 7 by results on samples of methyl linolenate diluted with various amounts of methyl myristate.

Temperature affects both the onset time and the extent of yellowing (Figure 8) and must be controlled for accurate results. A temperature of 50°C. was selected as being most appropriate from the standpoint of convenience and accuracy.

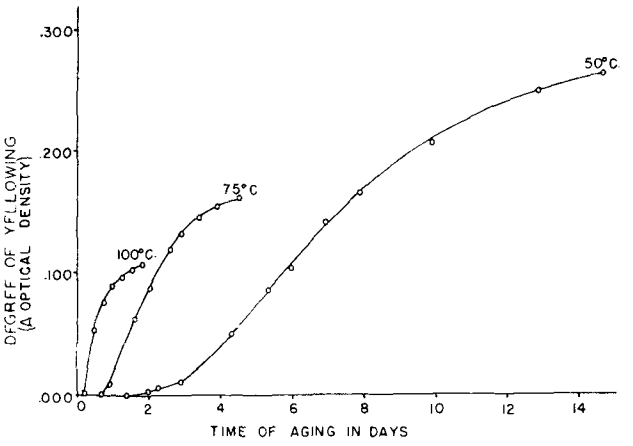

FIG. 8. The effect of temperature on the yellowing of films of methyl esters of linseed oil in the accelerated test.

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