

ments were made for any deviations introduced by the volume of oil or oil residue, and calculations were made in the following manner: $\mu\text{g } \gamma\text{-tocopherol originally present in sample aliquot} = (A_1) (\mu\text{g } \gamma\text{-tocopherol added per aliquot}) / (A_2 - A_1)$; where A_1 is the absorbance of corn oil and A_2 is the absorbance of corn oil plus added γ -tocopherol. Kaunitz and Beaver (7) used similar calculations.

Application of the Method. The method has been applied successfully to the analysis of a number of corn inbreds and crosses and has served to show that a several-fold range exists in tocopherol content. Some examples are shown in Table III. It has been observed that the absorbance drift usually falls close to the arbitrary value of 10% suggested by Baxter.

Summary

A method has been developed to overcome difficulties in the analysis of corn oil for tocopherol. The poor recovery of tocopherol added to corn oil prior to the Parker-McFarlane treatment does not occur with the present method, and the absorbance drift which occurs when crude corn oil reacts with iron and dipyrindyl is largely eliminated.

The interfering substance responsible for the drift did not distill appreciably in a molecular still at 170°C., and attempts to remove it from the oil by selective adsorption or solvent extraction were unsuccessful. It was extractable with dilute potassium hydroxide from a hexane solution of the oil and could be transferred from the alkaline extract to benzene. If the alkaline extract were acidified and then shaken

with benzene, a much larger amount of the substance could be transferred. Extracts containing the substance showed strong absorption at wave-lengths between 290 and 315 millimicrons.

Alkali treatment of the oil and subsequent adsorption on diatomaceous earth reduced the content of interfering constituents to permit analyses for total tocopherol by the Emmerie-Engel method. By using this method, it was found that the oil from different corn inbreds and crosses varied over a six-fold range in tocopherol content.

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The Use of Coloring Ingredients in Fatty Food Products. Their Physiology, Chemistry, and Stability

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THERE IS RELATIVELY little structural similarity in the chemical structures of the four coloring materials, ethyl bixin, β -carotene, Yellow AB, and Yellow OB, to account for the similarity of visible and ultraviolet spectra (except for double-bond conjugation and the similarity of Yellows AB and OB).

Table I shows that other physical properties of the compounds are equally dissimilar and presents the relative solubilities of these compounds.

On turning to the coloring values of these compounds, there is a rather wide variation in amounts of the pure substance necessary to color a ton of margarine. Based on extinction coefficients, Espoy and Barnett (6) reported the following equivalence:

1 g. β -carotene \equiv 4 g. FD&C Yellows 3 and 4 (mixed) \equiv 0.7 g. ethyl bixin.

On the basis of actual visual examination they reported the following ratios:

1 g. β -carotene \equiv 3.3 g. FD&C Yellows 3 and 4 (mixed) \equiv 0.5 g. ethyl bixin.

Analyses of many samples have shown there was up to 30% variation in the amount of color present; this has been confirmed by our experience with the

quantity of color specified by various manufacturers (Table I).

Figure 1 shows the ultraviolet spectra of the commercial colors as determined in our laboratories. These data agree with curves reported by Espoy and Barnett (6) in 1955.

Stability Studies

Until a short time ago fat-soluble annatto colors were prepared by extracting the fat-soluble color

TABLE I

	Ethyl Bixin	β Carotene	Yellow AB	Yellow OB
Empirical formula	C ₂₇ H ₃₄ O ₄	C ₄₀ H ₅₆	C ₁₆ H ₁₈ N ₃	C ₁₇ H ₁₈ N ₃
Molecular weight	422.54	536.85	247.29	261.31
Melting point	149°C.	181-182°C.	102-104°C.	125-126°C.
Form	Red rhombic crystals	Red rhombic leaflets, deep purple prisms	Orange powder, orange-red platelets	Orange powder, orange-red platelets
Solubility				
Water	I	I	I	I
Ethanol	S1.S.	I	S	S
Ethyl ether	S1.S.	S	S	S
Hexane		S		
Acetone	S	S	S	S
C ₆ H ₆	S	S	S	S
CCl ₄	S	S		
Vegetable oils	S1.S.	V. S1.S.	S	S
Experience use level, g./ton	2.7-3.5	7.2	10-12*	10-12*

*Assuming equal parts of each are used according to usual practice.

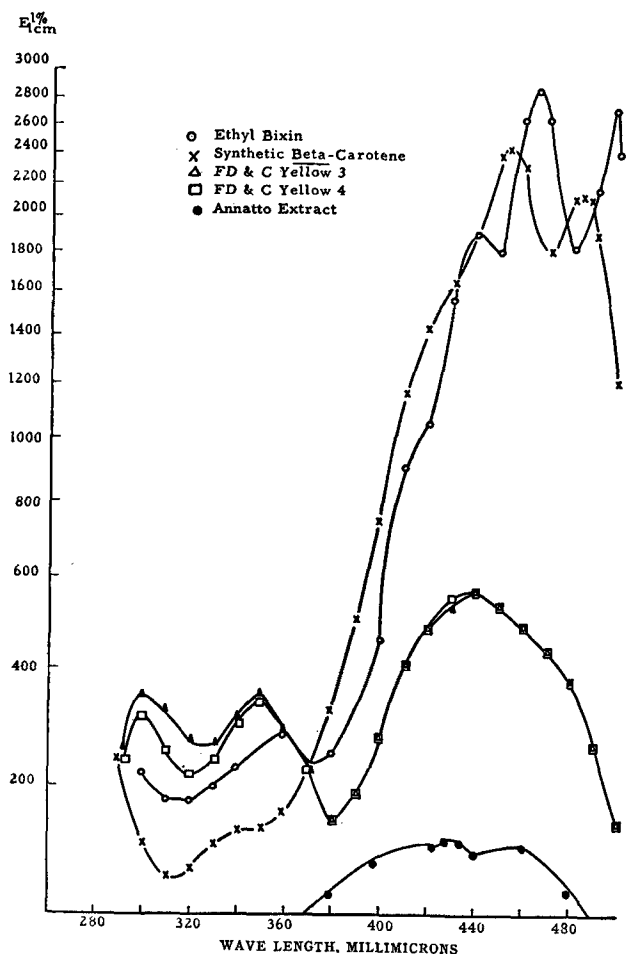


FIG. 1. Ultraviolet spectra of crystalline ethyl bixin, crystalline synthetic *beta*-carotene, crystalline FD&C Yellows 3 and 4, and annatto extract; solvent, carbon tetrachloride.

fraction from annatto beans. These color concentrates were not completely suitable, owing to standardization difficulties. Climatic, temperature, and soil changes resulted in variations in color intensities and shading characteristics and are variations to be expected from any natural product. Barnicoat (3, 4) as well as various margarine technologists (1) have reported that these annatto colors frequently produced red and other "off-colors" during storage in finished margarines and dairy products.

The structural formula of bixin, the principal color component of annatto, contains nine double bonds. Viebrock (17), in oxidation studies, reported that two of these double bonds are especially susceptible to oxidation, resulting in a deep red-colored compound. Further tests indicated that norbixin and methyl bixin behave in a similar manner; however the oxidation rate apparently is much slower. Iverson (9) reported that spectral examinations of freshly extracted annatto colors, heated samples of annatto colors, and commercial annatto butter color preparations showed the presence of various amounts of bixin as well as various transformation products of bixin. The transformation products had varying tinctorial properties, from red through orange and yellow and finally to colorless compounds.

Based on these findings, a more standardized and stable form of annatto color would prove more desirable for coloring fatty foods. In order to overcome

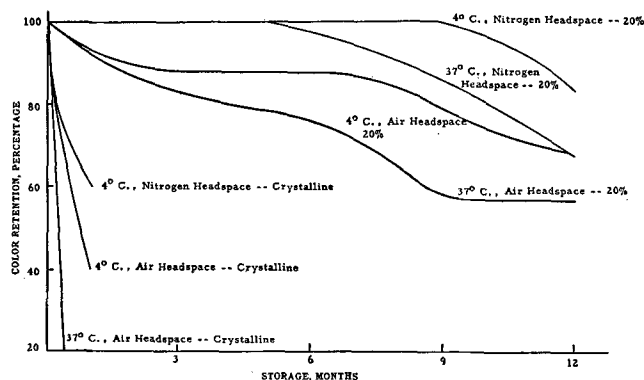


FIG. 2. Color retention (expressed as percentage of initial $E_{1\text{cm}}^{1\%} = 2930$) of crystalline ethyl bixin and color retention (expressed as percentage of initial $E_{1\text{cm}}^{1\%} = 600$) of 20% suspension ethyl bixin in cottonseed oil during varying storage conditions.

these tinctorial variations, ethyl bixin and several forms of *beta*-carotene were evaluated and found to be acceptable as margarine colorants. Ethyl bixin is a color-standardized product which eliminates color variations resulting from natural product variation. In addition, it is a relatively stable product which is more resistant to oxidation and which does not produce "off-colors" in stored margarine. Synthetic *beta*-carotene and highly refined forms of natural *beta*-carotene concentrates are equally acceptable for this use.

Pure crystalline ethyl bixin is an extremely unstable compound even when stored under an inert atmosphere with refrigeration, as shown in Figure 2. In addition, the relatively low order of solubility of this compound is a problem because of the short agitation time prescribed before the margarine enters the churn and the Votators. Therefore a fine crystalline product suspended in a protecting medium is needed. This condition is also true of pure crystalline *beta*-carotene.

Microcrystalline suspensions of ethyl bixin or synthetic *beta*-carotene in vegetable oils were prepared. The solubility rates were improved by this prepara-

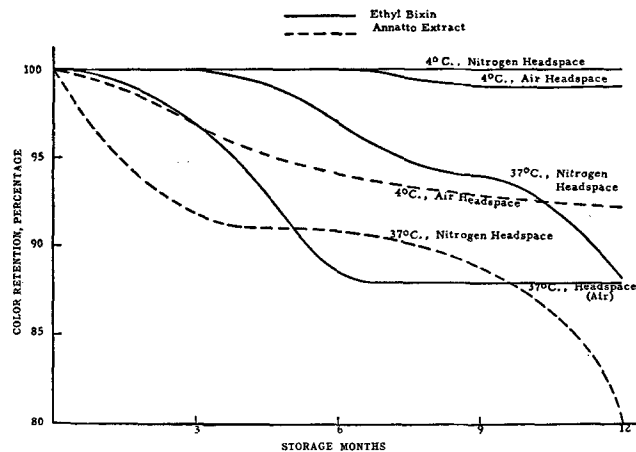


FIG. 3. Color retention (expressed as percentage of initial $E_{1\text{cm}}^{1\%} = 75$) of 2.5% suspension ethyl bixin with added vitamin A in cottonseed oil (batch-size) and of annatto extract with added vitamin A in cottonseed oil (initial $E_{1\text{cm}}^{1\%} = 103$; batch-size) during varying storage conditions.

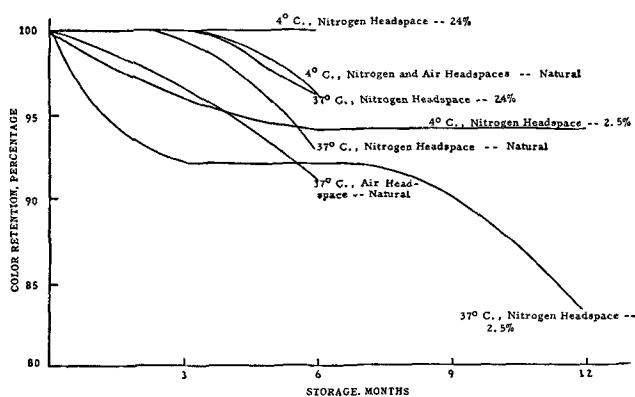


Fig. 4. Color retention (expressed as percentage of initial $E_{1\%}^{1\text{cm}} = 40$) of 2.5% suspension synthetic *beta*-carotene with added vitamin A in cottonseed oil (batch-size), of 24% paste synthetic *beta*-carotene in cottonseed oil (initial $E_{1\%}^{1\text{cm}} = 600$), of natural *beta*-carotene solution (initial $E_{1\%}^{1\text{cm}} = 9.6$), during varying storage conditions.

tion, and the prescribed quantity of color could be dissolved in warm margarine oils with less than five minutes of agitation time (16). Figures 2, 3, and 4 show the relative storage stability of a 20% oil suspension of crystalline ethyl bixin, an annatto extract in oil, and a 2.5% oil suspension of ethyl bixin, and oil suspensions of synthetic crystalline *beta*-carotene under air or nitrogen headspaces. By preparing the suspension in oil, the color crystals were protected from atmospheric oxidation. For the margarine industry these products packed with an air headspace have suitable stability time; however, by using an inert gas in the headspace of the packages, stability is markedly improved. Packaging with a nitrogen headspace and storing under refrigeration provides ideal stability for these compounds.

Metallic Ion Contamination. Metallic ions play an important role as pro-oxidants in fats and oils. In preparing a microcrystalline suspension, milling is necessary to reduce crystal size, and high quality, stainless steel equipment is necessary. Stability studies were made on milled samples containing added contaminants of metallic copper and iron. Figure 5

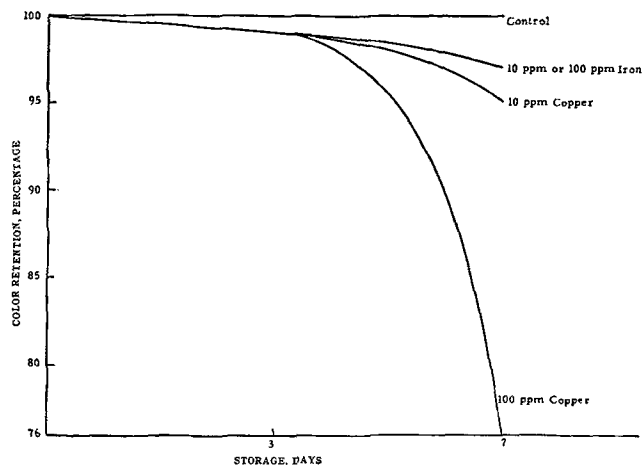


Fig. 5. Color retention (expressed as percentage of initial $E_{1\%}^{1\text{cm}} = 75$) of 2.5% suspension ethyl bixin in cottonseed oil and of 20% suspension ethyl bixin in cottonseed oil (initial $E_{1\%}^{1\text{cm}} = 600$) with added copper and iron, stored at 45°C.

shows loss in tinctorial properties of 2.5% and 20% oil suspensions of ethyl bixin, caused by isomerization of the compound when copper and iron were added.

These tests confirm previous reports that copper is a much more active pro-oxidant than iron (11).

Beta-Carotene Coloring Suspensions. *Beta*-carotene is available in a variety of physical forms for food coloring purposes. Synthetic *beta*-carotene, highly refined forms of natural *beta*-carotene, and other natural carotenes, which contain small quantities of xanthophylls carried over during processing from the source material, were tested for color stability and tinctorial properties.

Previous stability studies (5, 13-15) of carotene in various suspending mediums showed that a number of factors were responsible for the decomposition of carotene. Carotene extracted from carrots by vegetable oils showed an 8% loss after storage for four months at room temperature. Identical samples lost 23% when stored for four months and exposed to sunlight. All test samples were stored under carbon dioxide. As expected, ultraviolet rays are detrimental to the stability of *beta*-carotene (14).

Reported stability studies on natural carotene in various oils demonstrated the effects of these oils on the carotene. After 37 weeks of storage in the dark at 0 to 5°C. the carotene content of the oil solutions showed a 14% loss in cottonseed oil, 23% loss in apricot kernel oil, 25% loss in olive oil, and 30% loss in sunflower seed oil. Variations in the acid number, oxidation number, and iodine value of the oils used did not affect the loss of carotene (15). However mixtures of carotene (*beta* and *alpha*) when stored with crude, refined, or hydrogenated peanut oils at 37°C., indicated highest loss of carotene in refined oil and lowest loss in hydrogenated oil (13). This experiment suggested that the loss was catalyzed by peroxides contributed from the oil fraction. Again acid values and iodine number variations had no effect on the carotene stability. Earlier work (5) on this problem indicated that refined cottonseed oil was a superior solvent for carotene at 4°C. This study concluded that, although the acidity of the oil may influence the stability, it is not necessarily a major factor. Carotene losses were higher in corn oil with an acid number of 0.2 than in coconut oil with an acid number of 19.0; however carotene losses were highest in wheat germ oil having an acid number of 40.

Various forms of carotenes have been tested in our laboratories for stability under various conditions. During recent years the so-called batch-size cans containing sufficient color and vitamin A for one batch of margarine have become increasingly popular. The greatest demand for these colors in oil comes from the margarine industry, and stability studies were conducted on these mixtures. Synthetic *beta*-carotene, natural *beta*-carotene, natural *beta*-carotene (containing approximately 12% xanthophylls, 62% *beta*-carotene, and 26% *alpha*-carotene), and a mixture of ethyl bixin and the natural *beta*-carotene containing xanthophylls and *alpha*-carotene were each mixed with preformed vitamin A and suspended in cottonseed oil in batch-size proportions. The mixture of natural *beta*-carotene and ethyl bixin was prepared to provide a specific color balance in finished margarine. These samples were stored under air and nitrogen atmospheres at 37°C. and 4°C. to determine the

color stability of these mixtures. This study showed that natural *beta*-carotene and the mixture of natural *beta*-carotene and xanthophylls were stable. Results with synthetic *beta*-carotene are shown in Figure 4.

All of these coloring compounds when stored with the usual precautions (refrigeration and sealed packages) are relatively stable. All mixtures showed the best stability of carotene when stored under nitrogen at 4°C. Losses of 3 to 10% of the color after six months' storage are not highly significant, considering an inherent error of 1 to 5% in the assay.

Coloring Suspensions in Margarine. The stability of the color in the consumer product is of prime importance to the margarine manufacturer since he is responsible for the saleability and consumer acceptance of his product. Margarines were colored with ethyl bixin, with *beta*-carotene (natural or synthetic), and with a mixture of natural *beta*-carotene (containing xanthophylls and *alpha*-carotene) and ethyl bixin to the usual tinctorial level. These products were fortified with 15,000 U.S.P. units of vitamin A per pound. The margarine samples were held at 140°F. for six hours; the samples were emulsified, cooled, recrystallized, and stored at 70°F. for stability studies. The temperature and holding time were selected to simulate the extreme limits reported in the industry. A margarine sample containing equal parts of

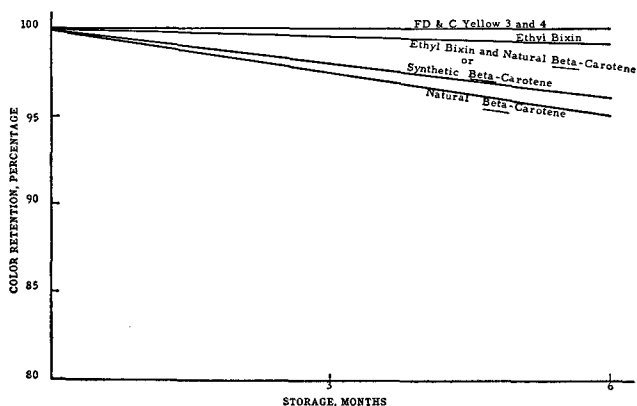


Fig. 6. Color retention (expressed as percentage of initial optical density = 1.00) of margarines colored with ethyl bixin, natural *beta*-carotene, synthetic *beta*-carotene and a mixture of natural *beta*-carotene and ethyl bixin during storage.

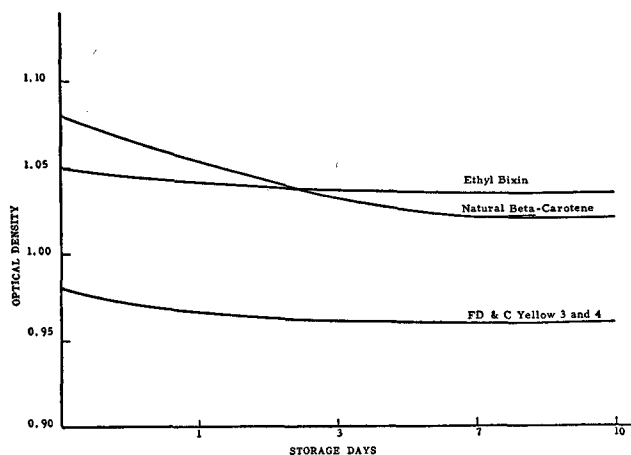


Fig. 7. Color retention of margarines colored with ethyl bixin, natural *beta*-carotene, and FD&C Yellows 3 and 4 during exposure to daylight.

FD&C Yellows 3 and 4 was used as a known stability standard.

Figure 6 shows that all samples had good color stability, and there was 98% retention of vitamin A after storage for six months. Melnick *et al.* (12) reported good stability of vitamin A and natural *beta*-carotene in margarine under storage at 74°F. for one month.

Ultraviolet Decomposition. The ultraviolet component in daylight produces oxidation in fats, oils, and vegetable colors. Color fade tests were made by using quarter-pound prints of commercially colored margarine (natural *beta*-carotene, FD&C Yellows 3 and 4, and ethyl bixin) and were exposed to daylight. These tests attempted to simulate household conditions. Previous studies (10) of margarine colored with *beta*-carotene indicated good carotene and vitamin A stability under these conditions.

Figure 7 shows relatively no loss of color in margarines colored with natural *beta*-carotene, FD&C Yellows 3 and 4, or ethyl bixin after 10 days of exposure to daylight. All samples had a slight surface darkening, approximately $\frac{1}{8}$ in. deep.

To determine if pH was the cause of darkening, commercial margarines colored with a mixture of natural *beta*-carotene and ethyl bixin and with FD&C Yellows 3 and 4 were tested. A similar darkening effect occurred in margarines with pH ranging from 4.5 to 6.5, confirming work by Antila (2).

The problem of surface darkening in colored margarines is rather prevalent throughout the industry. Previous investigations (1, 8) have suggested this effect may be caused by surface dehydration, resulting from permeability of packing materials, by a preponderance of hardened oils, by crystallization in the container, and by excessive working of the crystalline product.

Margarines, commercially prepared and colored with FD&C Yellows 3 and 4 or with a mixture of natural *beta*-carotene and ethyl bixin, were stored at 80°F. in darkening studies. The prints were wrapped in parchment in an accelerated temperature study.

Each month surface scrapings of $\frac{1}{8}$ in. from the prints were melted, and optical densities were determined on the oil fraction to measure any variation in color or shift in peak wavelength. No variations in optical density were detected; however a visual surface color darkening occurred on all prints. Comparisons were made on cut and uncut surfaces. The over-all color was appreciably darker in all prints; however no off-colors, such as green or red tints, were detected when the prints were compared against controls stored at -20°F.

Deep-Fat Frying: Oils. Our laboratories studied the stability of color in fatty products exposed to high heats. Highly colored popping oils and hydrogenated fats are used not only for the production of edible and nutritious food but also for aesthetic color which they impart to the products in which they are used. Various colors were added to liquid oils and hydrogenated fats, and the stabilities of these colors were evaluated under normal use conditions.

Samples of cottonseed oil were colored to 20 Red Lovibond, using synthetic *beta*-carotene, ethyl bixin, and FD&C Yellows 3 and 4. These colored oils were used to pop popcorn under normal household conditions. Results showed synthetic *beta*-carotene at

30 mg. per pound of oil and ethyl bixin did not survive the high-heat and moisture conditions encountered in the popping operation. The desired yellow color usually associated with popcorn was not achieved. When the level was raised to 60 mg. of *beta*-carotene per pound of oil, the popcorn had a color about that of the sample prepared using FD&C Yellows 3 and 4. With a level of 75 mg. of *beta*-carotene per pound of oil further increase in color intensity was obtained while a level of 120 mg. of *beta*-carotene per pound of oil yielded a very good butter-yellow color.

Samples of cottonseed oil colored with synthetic *beta*-carotene or ethyl bixin also were studied, using an oil colored with FD&C Yellows 3 and 4 as a control. The oils were heated to 365°F. (usual deep-fat frying temperature) and were held at this temperature for 10 min. The results of these tests (Figure 8) indicate again that all colors except FD&C Yellows 3 and 4 isomerize and decolorize at these high temperatures.

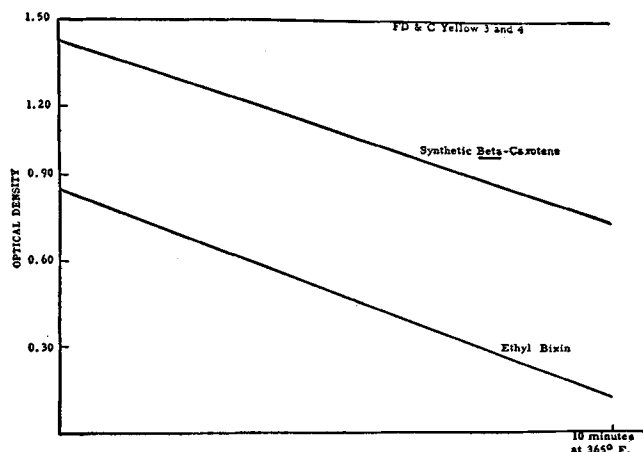


FIG. 8. Color retention of cottonseed oils colored with ethyl bixin, synthetic *beta*-carotene, and FD&C Yellows 3 and 4 heated at 365°F. for 10 min.

Deep-Fat Frying: Shortening. Samples of hydrogenated shortening composed of a mixture of soya bean and cottonseed oils were colored with synthetic *beta*-carotene, ethyl bixin, and FD&C Yellows 3 and 4. These samples were held at 375°F. for one hour to simulate the extremes of household, deep-fat frying practices. Figure 9 shows the instability of ethyl bixin and synthetic *beta*-carotene as coloring agents in frying fats.

Shortening. Although baking operations involve rather high temperatures, the carry-over of color when using carotenoid-colored shortenings was quite good. Silver layer cakes were used because this recipe does not include egg yolks, thus eliminating one pos-

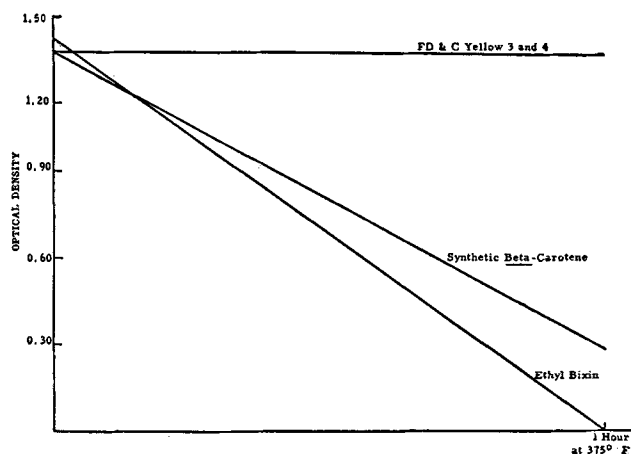


FIG. 9. Color retention of hydrogenated shortenings colored with ethyl bixin, synthetic *beta*-carotene, and FD&C Yellows 3 and 4 heated at 375°F. for one hour.

sible color variation. The cakes were baked at 350°F. for 25 min., and cut surfaces were visually examined and compared with a control cake prepared with the same recipe using uncolored shortening. Color carry-over was excellent when *beta*-carotene or ethyl bixin was used to color the shortening. Similar results have been reported by Fellers (7).

Summary

Vegetable oil suspensions of ethyl bixin and *beta*-carotene, to which vitamin A may be added, have excellent stability and are readily adaptable for use in coloring margarine and other fatty food products. Based on normal finished product turnover these colorants maintain adequate stability. In frying fats and popcorn oils only FD&C Yellows 3 and 4 are completely stable.

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