Reaction of Carotenes in Palm Oil with Acid

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Carotenes in palm oil reacted with strong acid under nonaqueous conditions to produce blue-green cations that initially absorbed with a broad maximum at approximately 900 nm. These cations were postulated to be formed from the protonation of the α- and β-carotene at the seventh carbon atom, with the charge delocalized between the 8 and 9 conjugated double bonds. They were unstable at room temperature and may, by isomerization and oxidization, form carotenoid compounds with shorter conjugation. The resulting carotenoids can be protonated further to form cations, which absorbed between 500–900 nm, with λ_{max} of approximately 800 nm. The transient blue-green color observed in partially bleached palm oil is likely due to the presence of these cations.

KEY WORDS: Acid, carotenes, cations, palm oil.

Carotenoids are present in palm oil to an extent of about 700 ppm and in varying amounts in other vegetable oils (1). Thus, the bleaching of vegetable oils, in particular palm oil, essentially consists of the removal of the brightly-colored carotenoids, either by adsorption or by heat treatments. Acid-activated bleaching clays are commonly used in adsorptive bleaching. During the physical refining of palm oil, the intermediate oil, after the initial adsorptive bleaching but before deodorization, has reduced red and yellow colors, but an increased blue color (2). Transient blue-green colorations have also been noted when acid-activated rice hull ash was added to soy oil in hexane miscella (3) and to palm oil (4) miscella in hexane. A similar phenomenon has also been reported when acid-activated bleaching clays and some other solids were used as adsorbents for β-carotene solutions (5–7). No comment regarding the nature of the coloration was made in these reports. The development of a blue-green coloration in vegetable oils with added carotene on standing at low temperature was investigated in some detail in the late 1960s (8–10). A number of oxygenated carotenoid compounds were isolated, but no physical evidence, other than the color formation, was presented for the intermediates. Because vegetable oils are mainly used for edible purposes, it is important to know as much as possible about the nature of color formation during their refining.

The common denominators in the observations mentioned above are the presence of carotenes and acidity either on the solid absorbents (3–7) or in trace amount in the oils (8–10). We thus proceeded to investigate the effect of acid on palm oil miscella.

EXPERIMENTAL PROCEDURES

Crude palm oil (CPO) was obtained from Palmex Industries (Prai, Penang, Malaysia). The total carotene content was determined to be 534 ppm by an ultraviolet (UV)-visible spectrometer with an all-*trans* β-carotene (Sigma Chemical, St. Louis, MO) as standard.

CPO solution was prepared by stirring 2.0 g of CPO in 20 mL diethyl ether (analytical reagent; BDH, Poole, United Kingdom), which had been previously dried by activated molecular sieve and degassed. This solution was kept in the dark to avoid deterioration. Acid solution was prepared by adding 1 mL of concentrated H_2SO_4 (Analyzed reagent; J.T. Baker, Phillipsburg, NJ) dropwise with stirring into 39 mL of dried and degassed diethyl ether, which was cooled in a water bath. Acid solutions of different concentrations were prepared by adding the required volume of concentrated acid to diethyl ether, such that the total volume was 40 mL.

The reaction was carried out by adding varying amounts of the acid solution to 5 mL of CPO solution at 27 ± 1°C and stirring. The spectra of the resulting mixtures were recorded in a UV-visible spectrometer (U2000 Hitachi; Hitachi, Tokyo, Japan) within a range of 200–1000 nm.

The carotene in palm oil was extracted and concentrated before being analyzed by the procedure of Ng and Tan (11), except that the high-performance liquid chromatography (HPLC) system used was an HP 1090 by Hewlett-Packard (Palo Alto, CA) with a 25 × 4.6 mm Rainin Microsorb ODS Hypersil column, and that the solvent mixture used was 60% acetonitrile, 35% methanol and 5% methylene chloride at a flow rate of 1.8 mL/min.

RESULTS AND DISCUSSION

On mixing 5 mL crude palm oil in diethyl ether miscella with 40 mL of H_2SO_4 solution in the same solvent at room temperature, a blue-green coloration was immediately observed. The UV-visible spectra of this mixture was recorded at different time intervals. These, together with the original palm oil miscella adjusted to the same concentration, are shown in Figure 1. Immediately after mixing, the λ_{max} for the carotene was lowered by 3 nm, with a loss in resolution and decreased intensity. At the same time, the peak at 268 nm increased in intensity. A new broad absorption at λ_{max} 900 nm was observed. On standing, the intensities of the absorption maxima due to carotene decreased slightly over 4 min, while the broad maximum at 900 nm decreased by almost half. At 30 min after mixing, this broad maximum completely disappeared, and absorption appeared between 500–900 nm with λ_{max} at 800 nm. On standing for 2 h, the absorption maxima, due to carotene, also completely disappeared. The intensity of the absorption between 500–900 nm increased. The mixture remained blue-green. The peak at 268 nm remained relatively unchanged. This absorption was due to the presence of a triene chromophore, which may have been formed from dehydration of diene peroxide originally present in the CPO by the concentrated sulfuric acid. This was demonstrated by the increase in its absorption when H_2SO_4 was added to aged refined palm oil that contained negligible carotene.

On mixing the same CPO in either miscella with 40 mL of acid solution of different concentration, the broad absorption at 900 nm, measured after 1 min, increased with concentration but decreased slightly at higher concentration (Fig. 2). The disappearance of the carotene absorption peak was also observed to occur more rapidly.

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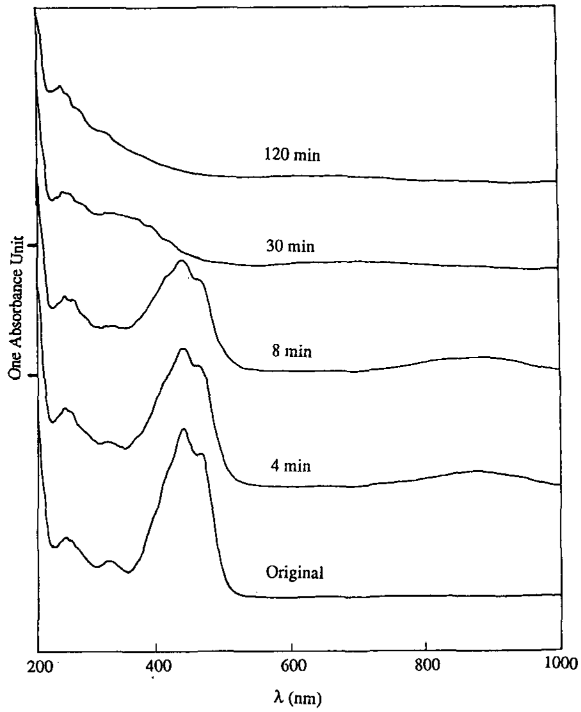

FIG. 1. Ultraviolet-visible spectrum of original palm oil miscella and that of the reaction mixture at different time intervals after the addition of 40 mL of H_2SO_4 solution to 5 mL of crude palm oil solution.

On addition of an equal volume of water to the blue mixture formed 10 min after mixing, the blue color faded and was replaced by a yellowish-orange solution in the ether layer, which resembled the original palm oil solution. To make sure that the yellow recovered color was not due to the unreacted carotene, addition of acid solution to the mixture before addition of water was continued until two layers were formed. The top was an oily, yellow-orange layer, and the bottom layer was blue. After separation, the blue bottom layer gave spectrum A in Figure 3. This solution was then added to an equal volume of water and shaken. A colorless aqueous layer and a yellow ether layer were formed. The UV-visible spectrum of the yellow ether solution showed an absorption maximum in the carotene region (spectrum C in Fig. 3), but with greatly reduced resolution. The absorption peak at 268 nm was also increased compared to that of the original CPO solution. When acid solution was added to this yellow solution, greenish-blue coloration was again formed with absorption between 500–900 nm (spectrum D in Fig. 3), while the carotene absorption was much reduced.

The HPLC chromatogram of the extract of the products of the reaction is shown, together with the carotene extract of the original palm oil solution, in Figure 4. The chromatogram of the carotene in the palm oil closely resembles that reported earlier (11). Other than the major components of α and β carotenes, and the minor γ, ζ and ε carotenes, the remaining peaks were identified as oxygen-containing xanthophylls, including dehydroretinal, canthaxanthin, carotenal and six epoxides of α or β carotenes. Comparison of the chromatograms of the original

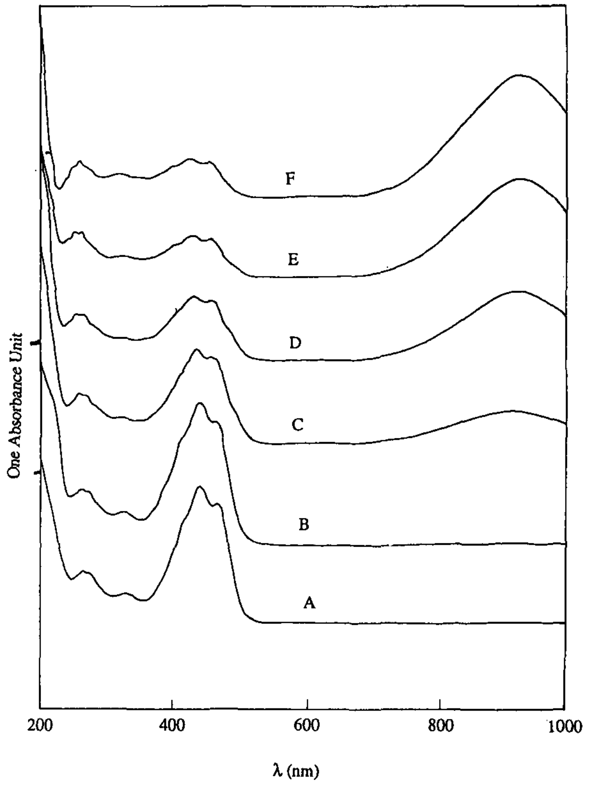

FIG. 2. Ultraviolet-visible spectrum of the mixtures of palm oil miscella and H_2SO_4 solution of different concentrations one minute after mixing. A, No H_2SO_4 solution was added; B, C, D, E and F, with 40 mL of 1.25, 3.75, 6.25, 8.75 and 11.25 vol% of H_2SO_4 solution, respectively.

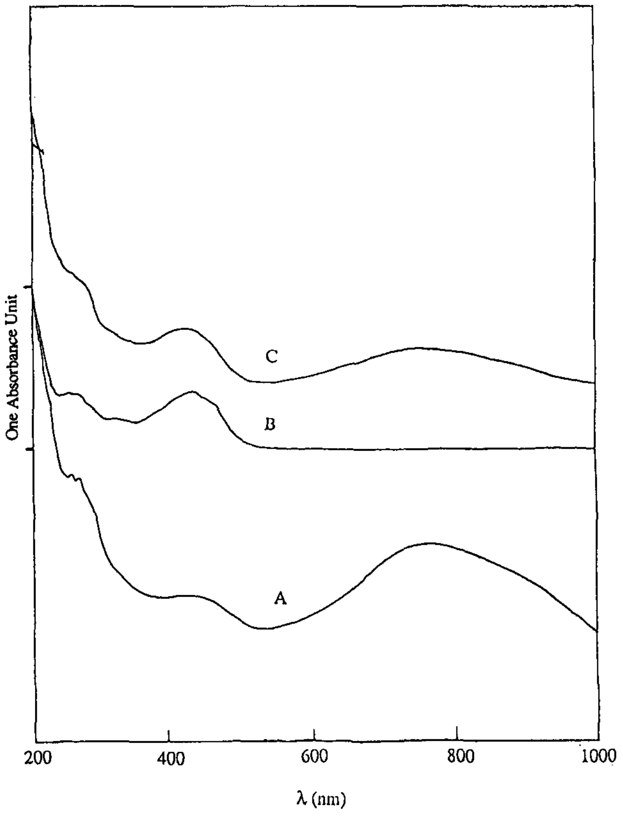

FIG. 3. Ultraviolet-visible spectrum of palm oil miscella after mixing with acid solution and water. See text for full discussion.

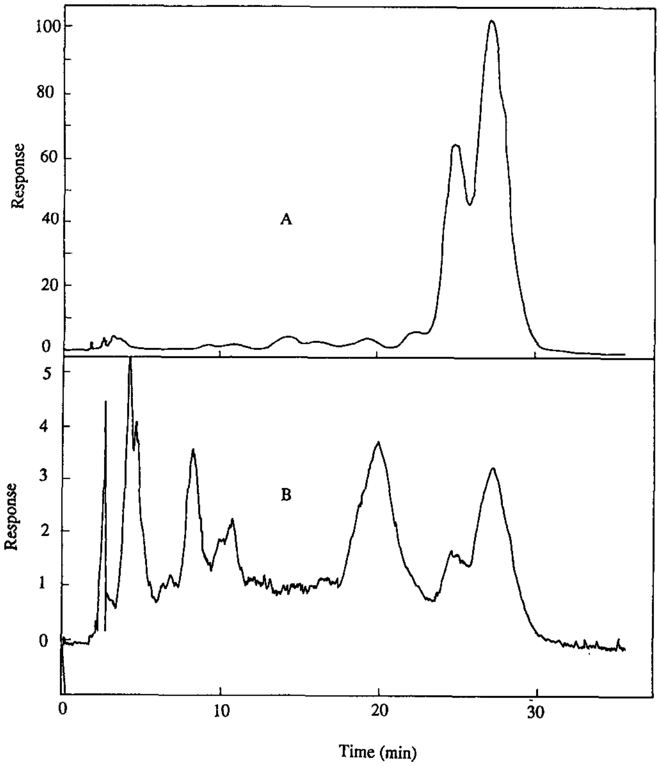

FIG. 4. The high-performance liquid chromatography chromatograms of carotene extract of (A) original palm oil solution and (B) reaction products extracted from the mixture of palm oil miscella and H_2SO_4 solution.

carotenoids and the products shows that the products consisted of isomerized and epoxidized carotenoids, while the original α and β carotenes' concentrations were greatly reduced. The identities of these products are currently being investigated.

These observations could be explained by the initial formation of carotene cations from the protonation of the carotenes in the palm oil, when a solution that contained carotene molecules was mixed with concentrated H_2SO_4. The formation of polyene cations has been well-documented, as in the Carr-Price reactions (12,13). The absorption maximum, λ_{max}, of these polyene cations depends on the number of conjugated double bonds, **n**, and can be approximately correlated by the equation (Ref. 12):

$$\lambda_{max} = 330 + 65.5\mathbf{n}\ \text{nm} \qquad [1]$$

The composition of the carotenes in Malaysian palm oil has been determined to be about 54% β-carotene, 36% α-carotene and other minor carotenoids (14). The carotenes in the CPO used consisted of the *trans*, as well as the *cis* isomers, as shown by the absorption maximum at 340 nm in the original solution (Fig. 1).

For the β-carotene, the most active position is at the 7 and 8 carbons (15). Thus, protonation at the seventh carbon in the chain will result in a carotene cation with $\mathbf{n} = 9$, which will absorb with a λ_{max} of approximately 920 nm. However, protonation of an α-carotene at the same position will result in a cation with $\mathbf{n} = 8$, which absorbs with a λ_{max} of approximately 900 nm, which likely resulted from the overlap of the absorptions of the two cations. The concentrations of these cations were dependent upon the concentration of the added acid, as shown by the increase in intensity of the absorbance as the acid concentration was increased. An equilibrium was presumably established between the carotene and the acid with the cations. These cations were rather unstable at room temperature because they decayed with time and were completely destroyed on standing within 1 h. They were also susceptible to attack by water or alkali; carotene molecules were again obtained. However, these recovered carotenes were not the original carotene molecules, as shown by the decreased resolution of the peaks in the 446 nm region and the HPLC chromatogram of the product (Fig. 4).

Although the species that absorbed at 900 nm were destroyed on standing, the solution remained blue due to the increase in absorbance at 800 nm, which was not initially observed. Subsequent to the initial protonation of the molecules at the seventh carbon position, the cations formed were susceptible to isomerization, to oxidation by traces of oxygen remaining or the concentrated sulfuric acid, and to nucleophilic attack by molecules present in the oil or moisture, resulting in molecules with shorter conjugation. These were likely to be protonated to cations that absorbed at a shorter wavelength.

Surface Brønsted acid sites on solid catalysts are well known as active sites for heterogeneously catalyzed reactions involving carbonium ions as intermediates. Polynuclear hydrocarbons, when adsorbed on acidic catalysts, produced blue-green cation radicals as a result of electron transfer between the molecule and the Brønsted acid sites (16–18). Adsorption of carotene on montmorillonite clay has been attributed to the formation of surface carotene cations by Sarier and Guiler (19) although in their paper, no physical evidence was presented. McWeeny (9) attributed the appearance of the green coloration in carotene-augmented vegetable oil to the reactions between the residual acid and the carotene epoxides, which were formed earlier. Taking these and other observations mentioned earlier into consideration, the following reaction scheme can be proposed:

$$\alpha \text{ and } \beta \text{ carotenes} + H^+ \rightleftharpoons \text{carotene cations } (\lambda_{max} \sim 900 \text{ nm})$$

$$\text{carotenoid cations} \rightleftharpoons H^+ + \text{oxidized, isomerized and other products}$$

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