

# The Mechanism of Color Reversion in Soybean Salad Oil

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An enzymatic reaction might be the major cause of color reversion in soybean oil. During the soybean flaking process, enzymatic reactions take place to convert  $\gamma$ -tocopherol to 5-(tocopheryloxy)- $\gamma$ -tocopherol ( $\gamma$ -TED), and then further convert  $\gamma$ -TED to the precursors which are the cause of color reversion. The variations of  $\gamma$ -tocopherol and  $\gamma$ -TED contents in soybean oil are accompanied by an inverse change in the precursors' concentration. Therefore, the contents of  $\gamma$ -tocopherol or  $\gamma$ -TED in crude soybean oil could be used as an index for predicting the color quality (color reversion) of soybean salad oil.

Almost all the precursors (such as tocored) were converted to color-reverted substances under catalysis after bleaching treatment, and they were converted back to the precursors during deodorization. Those precursors might be gradually reconverted into the color reverted substances during storage to cause the color reversion in soybean salad oil.

Refined soybean oil, usually sold under the name of salad oil, is a light-colored product when freshly prepared. However, a light yellow color develops during storage and the color will darken as time passes. This well known phenomenon is called color reversion, and has long been a problem for the vegetable oil industry.

Extensive studies have led to the identification of a wide variety of compounds as possible causative agents for the color reversion found in many different kinds of oils. For example, Tan et al. (1) reported that the specimens responsible for color development in heated palm oil are phenolic compounds. Swift et al. (2) reported that  $\gamma$ -tocopherol could be converted into chroman-5,6-quinone (tocored), causing the color reversion in cottonseed oil. Tomita et al. (3) first noted that the moisture content in soybean might be the major color reversion factor in soybean salad oil. Extensive studies of color reversion in soybean salad oil were made by Komoda et al. (4-7). According to their reports, the moisture content of soybeans (more than 12%) is a determining factor controlling color reversion in soybean oil. The amount of tocopherols present in the crude oil is also markedly affected by the moisture content in soybeans.

We report the results of studies on the cause of color reversion; the correlations of  $\gamma$ -tocopherol and  $\gamma$ -TED contents in soybean oil with the color reversion, and the mechanism of color reversion in soybean salad oil.

## MATERIALS AND METHODS

$\gamma$ -TED and 5-(tocopheryl)- $\gamma$ -tocopherol ( $\gamma$ -TBD) were donated by Yukihiro Ishilawa, Hiroshima Food Research Institute, Japan. Tocopherol isomers ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$

forms) were purchased from E. Merck. All solvents for HPLC analyses were of LC grade. The n-hexane used for extraction of soybean oil was of commercial grade. Unless otherwise specified, all other chemicals used were of reagent grade. Raw soybeans used were imported U.S. soybeans, FGIS Grade No. 2.

**General methods.** Moisture content was determined according to an AOCS method (8). Oil color was estimated by a Lovibond Automatic Tintometer. A one-inch cell was used to measure the crude and degummed oil, while a 5.25" cell was used in the measurements of neutralized, bleached and deodorized oil. The color reversion test was performed at room temperature.

**Raw soybean treatment.** Soybean samples (3.5 kg) were prepared for the studies. The desired moisture content (10-21%) of each sample was adjusted by mixing the soybeans with a calculated amount of deionized water. The soybeans were stored in an airtight glass bottle and allowed to equilibrate for one week with periodic shaking (twice per day). The treated soybeans were then processed according to the sequence of crushing, flaking, extracting and refining to make salad oil.

**Effects of storage temperature of soybeans on the Rm and the content of color-causing components ( $\gamma$ -tocopherol,  $\gamma$ -TED and tocored) in the corresponding crude oil.** Rm is defined as the highest red color value during color reversion in soybean salad oil. Samples of 3.5 kg of moistened soybean were stored at 60°C, 25°C or 6°C separately for one week, and then processed by crushing, flaking, extracting and refining the oil at room temperature. Each sample's moisture content was measured before processing. A parallel experiment using 20 g of moistened soybeans was carried out in which the storage, crushing, flaking, and extracting with n-hexane were all conducted under -20°C. The crude oils obtained from these samples were then analyzed and compared.

**Conversion of  $\gamma$ -tocopherol and  $\gamma$ -TED.** To an n-hexane solution containing  $\gamma$ -tocopherol (100 ppm) was added 3 g of flakes or 0.25 g crude soybean oil. After storage for two days, the solutions were analyzed for the change of  $\gamma$ -tocopherol, analogous experiments were carried out with solution containing  $\gamma$ -TED (55 ppm).

**Effects of different processing conditions of flakes on the Rm during color reversion in soybean salad oil.** The flakes obtained from soybeans with various moisture contents were either heated at 130°C for 20 min or moistened with deionized water, then heated at 130°C for 20 min. The treated flasks were then processed by the same extracting and refining procedures.

**Synthesis of tocored.** Tocored was synthesized according to the procedures reported by Frampton et al. (9).

**HPLC determination of tocopherol isomers,  $\gamma$ -TED, tocored and compound x.** Tocopherol isomers,  $\gamma$ -TED and tocored, present in the soybean oil sample prepared by mixing one g of soybean oil with 10 ml of

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LC grade n-hexane, were analyzed with a Waters high performance liquid chromatograph (Model 6000A) equipped with a Kratos Spectroflow 980 fluorescence detector, a Kratos Spectroflow 757 UV detector and a Zorbax Sil column (25 cm × 4.6 mm). The insoluble materials were removed by filtration through a Milipore FH membrane (0.5 mm) prior to injection. The flow rate of mobile phase was one ml/min, and the injection volume was 10  $\mu$ l. For the separation of tocopherols and tocored, the mobile phase was 2% isopropanol and 0.5% absolute ethanol in n-hexane. With a condition similar to that of Ishikawa (10), the  $\gamma$ -TED could be isolated with a mobile phase of 1.5% isopropylether in n-hexane. A mobile phase with 0.5% isopropanol and 0.5% absolute ethanol was used to separate the compound x in soybean oil. Fluorescent detection of tocopherol isomers and  $\gamma$ -TED was conducted at an excitation wavelength of 292 nm and an emission wavelength of 320 nm. Tocored and compound x were detected by UV absorptions at 275 nm and 460 nm, respectively.

## RESULTS AND DISCUSSION

Tocopherols have been suggested to be the causative substances for color development in vegetable oil (11-12). As shown in Figure 1 (A),  $\gamma$ -tocopherol is the major tocopherol present in soybean oil (13). Because  $\gamma$ -tocopherol is known to be a very reactive compound among the isomers of tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ) (14), the color reversion of soybean oil may be attributed to the oxidation of  $\gamma$ -tocopherol.

There are three known dimeric species of  $\gamma$ -tocopherol, i.e.,  $\gamma$ -TED and two isomers of  $\gamma$ -TBD. As shown in Figure 1 (B),  $\gamma$ -TED is the major dimer (ca. 99%) detected in soybean oil. This finding agrees with the data reported previously (10, 15, 16).

The effect of different storage temperatures of soybeans on the resulting Rm of the corresponding oil

samples and the contents of  $\gamma$ -tocopherol,  $\gamma$ -TED and tocored in these samples was examined first. As shown in Table 1, oil from soybeans stored at 60°C (A-1, A-2) gave the lowest Rm while those stored at room temperature (C-1, C-2) had the highest Rm. Table 1 also indicates that oil from samples stored at 60°C (A-1, A-2) and -20°C (D-1) show lower contents of tocored, while those stored at 25°C or 6°C were high in tocored content. The variation of the tocored content in different samples is always accompanied by a similar change in its  $\gamma$ -TED concentration. Analyses of the content of  $\gamma$ -tocopherol in these samples revealed that it is temperature dependent, albeit with a trend opposite that of the data of other columns in Table 1. Because the color reversion is directly related to the concentration of  $\gamma$ -tocopherol and its derivatives, the observed temperature dependence of their variation and the color intensity (Rm) as well, suggests that the key step in controlling the color conversion may be mediated by enzymes. Namely, the enzymatic reactions take place during the processing of soybean to convert  $\gamma$ -tocopherol to the color-causing products, and the corresponding enzymes may be inactivated at high temperature (60°C) or inhibited at very low temperature

TABLE 1

Effects of Storage Temperature of Soybean on the Rm During Color Reversion in Soybean Salad Oil and the Contents of the Components<sup>a</sup> in the Corresponding Crude Oil

No	M <sup>b</sup> (%)	T <sup>c</sup> (°C)	Rm (R)	$\gamma$ -Tocopherol (ppm)	$\gamma$ -TED (ppm)	Tocored (ppm)
A-1	20.3	60	1.9	633.0	18.2	35.5
A-2	18.8	60	1.3	689.6	17.6	33.6
B-1	17.3	6	4.8	251.5	98.7	62.5
B-2	17.3	6	3.6	203.6	77.0	65.5
C-1	17.3	25	8.2	241.1	66.9	57.1
C-2	17.8	25	6.2	203.1	78.7	53.9
D-1 <sup>d</sup>	16.4	-20	---	779.0	44.6	18.4

<sup>a</sup> $\gamma$ -Tocopherol,  $\gamma$ -TED and tocored.

<sup>b</sup>M, moisture content measured before processing.

<sup>c</sup>T, temperature for soybean storage (one week).

<sup>d</sup>20 g of soybeans stored, crushed and extracted under -20°C.

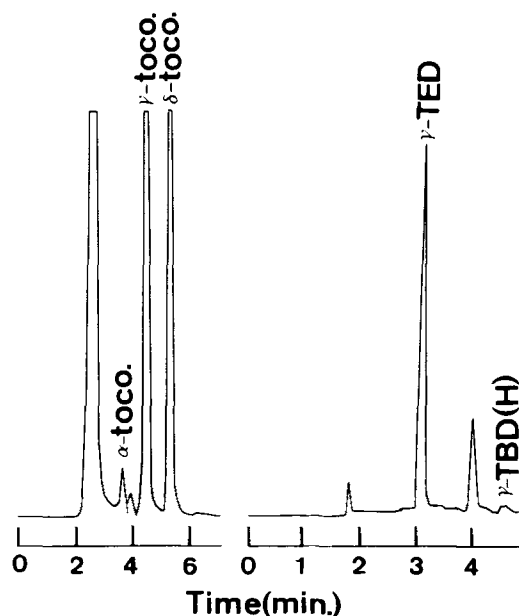


FIG. 1. HPLC chromatograms of  $\gamma$ -tocopherol and  $\gamma$ -TED in soybean oil. A, tocopherols; B, dimers of  $\gamma$ -tocopherol.

TABLE 2

The Conversions of  $\gamma$ -Tocopherol and  $\gamma$ -TED in n-Hexane Solution in the Presence of Flakes or Crude Oil

	No. <sup>a</sup>	$\gamma$ -Tocopherol (ppm)	$\gamma$ -TED (ppm)	Tocored (ppm)
Flakes <sup>c</sup>	A	nd <sup>b</sup>	nd	1.9
	B	0.4	13.5	12.9
	C	0.8	32.4	11.6
Crude <sup>d</sup> oil	A	7.3	5.6	4.1
	B	7.9	55.6	4.2
	C	97.0	7.8	4.6

<sup>a</sup>Added to 10 ml n-hexane solvent; B, added to 10 ml 55 ppm  $\gamma$ -TED n-hexane solution; C, added to 10 ml 100 ppm  $\gamma$ -tocopherol n-hexane solution.

<sup>b</sup>nd, Not detected.

<sup>c</sup>3 g Flakes.

<sup>d</sup>0.25 g Crude soybean oil.

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(sample D-1,  $-20^{\circ}\text{C}$ ). An oxidase-like enzyme (17) may be responsible for such conversion.

In an attempt to pinpoint the effects of the target enzymes responsible for the conversion of  $\gamma$ -tocopherol and  $\gamma$ -TED, soybean flakes and crude soybean oil were added separately into solutions of  $\gamma$ -tocopherol (100 ppm) or  $\gamma$ -TED (55 ppm) as shown in Table 2, near quantitative conversion (99.2%) of  $\gamma$ -tocopherol into  $\gamma$ -TED, tocored or other oxidation products was observed when  $\gamma$ -tocopherol (100 ppm) was incubated with soybean flakes. Addition of flakes to the  $\gamma$ -TED solution (55 ppm) also induced a significant conversion (75.5%). However, the efficacy of the crude oil was almost nil. These results were not surprising, because the target enzymes, if present in the flakes, were expected to be inactivated or removed during the oil refining process. These results also indicated that the key reaction or precursor formation of color reversion should take place prior to oil refining.

The effects of different processing conditions of flaking on the Rm during color reversion in soybean salad oil were then studied. As shown in Table 3, higher Rm values, i.e., greater color reversion, were found in soybean salad oil made from the flakes which had been heat treated or were moistened to higher moisture contents and then dried. These results suggest that the enzymatic conversion occurs during the flaking process, because the subsequent modification by changing the moisture content and/or heating the flakes showed no improvement on color reversion.

The oil produced from soybeans with high moisture contents required only a few days to develop color reversion, whereas the oil derived from soybeans with

low moisture content needed a much longer time. The higher the moisture content of soybeans, the higher the Rm and Ri of the soybean salad oil, (Ri is defined as the red color value of salad oil before color reversion). However, slight color reversion still occurred, even though the moisture content was below 10%. Komoda et al. (4) had reported that the tocopherol content in oils is inversely proportional to the moisture content in soybeans, i.e., the higher the moisture content in the soybeans, the less the tocopherol in the oil. This phenomenon correlated well with our results, based on the analyses of  $\gamma$ -tocopherol in soybean oils derived

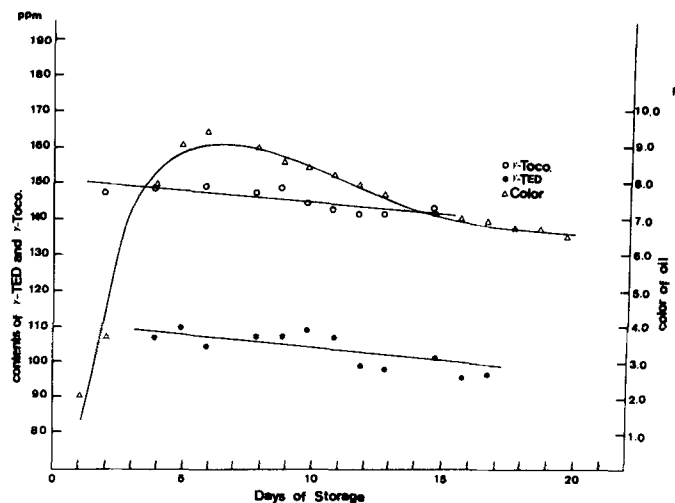


FIG. 2. The changes of  $\gamma$ -tocopherol and  $\gamma$ -TED contents in soybean salad oil during color reversion.

TABLE 3

The Effects of Different Processing Conditions of Flakes on the Rm During Color Reversion in Soybean Salad Oil

No.	Moisture (%)	Rm (R)
M-1	11.6	1.4
M-2	11.9	1.8
M-3	14.0	3.2
M-4	12.5 $\Delta$ 10.3	3.2
M-5	14.2 $\Delta$ 10.6	8.9
M-6	11.9 $\Delta$ 18.1 $\Delta$ 10.2	4.0

$\Delta$ , Flakes heated at  $130^{\circ}\text{C}$  for 20 min;  $\blacktriangle$ , moistened with deionized water.

TABLE 4

The  $\gamma$ -Tocopherol and  $\gamma$ -TED Contents in Soybean Oil From Soybeans with Different Moisture Contents during Refining Processes

Steps <sup>a</sup>	$\gamma$ -Tocopherol (ppm) <sup>b</sup>			$\gamma$ -TED (ppm) <sup>b</sup>		
	a	b	c	a	b	c
A	453.3	255.3	136.2	180.3	83.8	58.6
B	461.5	255.4	169.1	190.8	85.0	63.1
C	380.0	250.2	141.6	216.2	90.0	64.4
D	350.0	217.1	138.8	216.5	91.3	65.5
E	362.90	238.3	148.1	199.4	87.1	62.7

<sup>a</sup>A, Crude oil; B, degummed oil; C, neutralized oil; D, bleached oil; E, deodorized oil.

<sup>b</sup>Moisture contents of soybean: a, 11.1%; b, 14.9%; c, 18.2%.

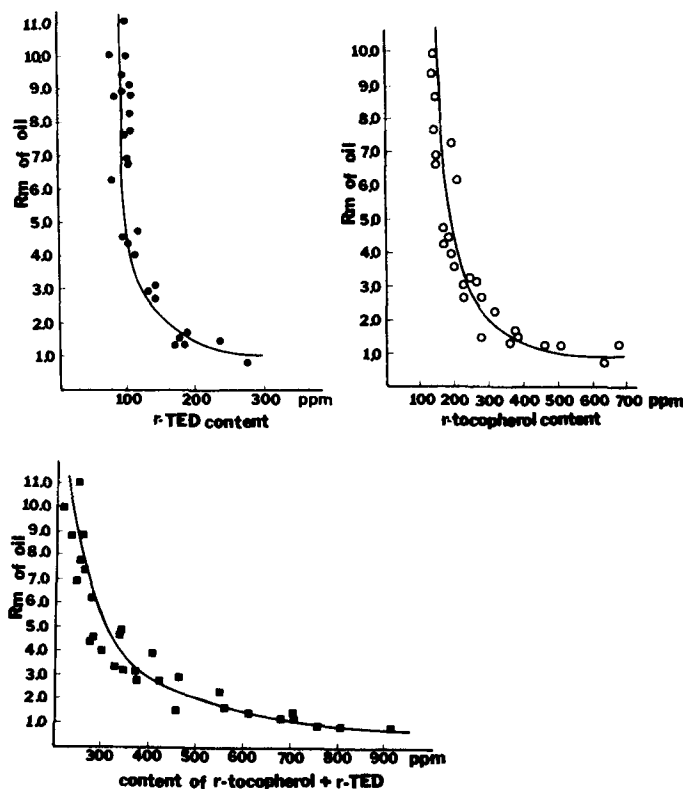


FIG. 3. The correlation of the contents of  $\gamma$ -tocopherol,  $\gamma$ -TED and both components in different batches of crude oil with the Rm of corresponding soybean salad oil during color reversion.

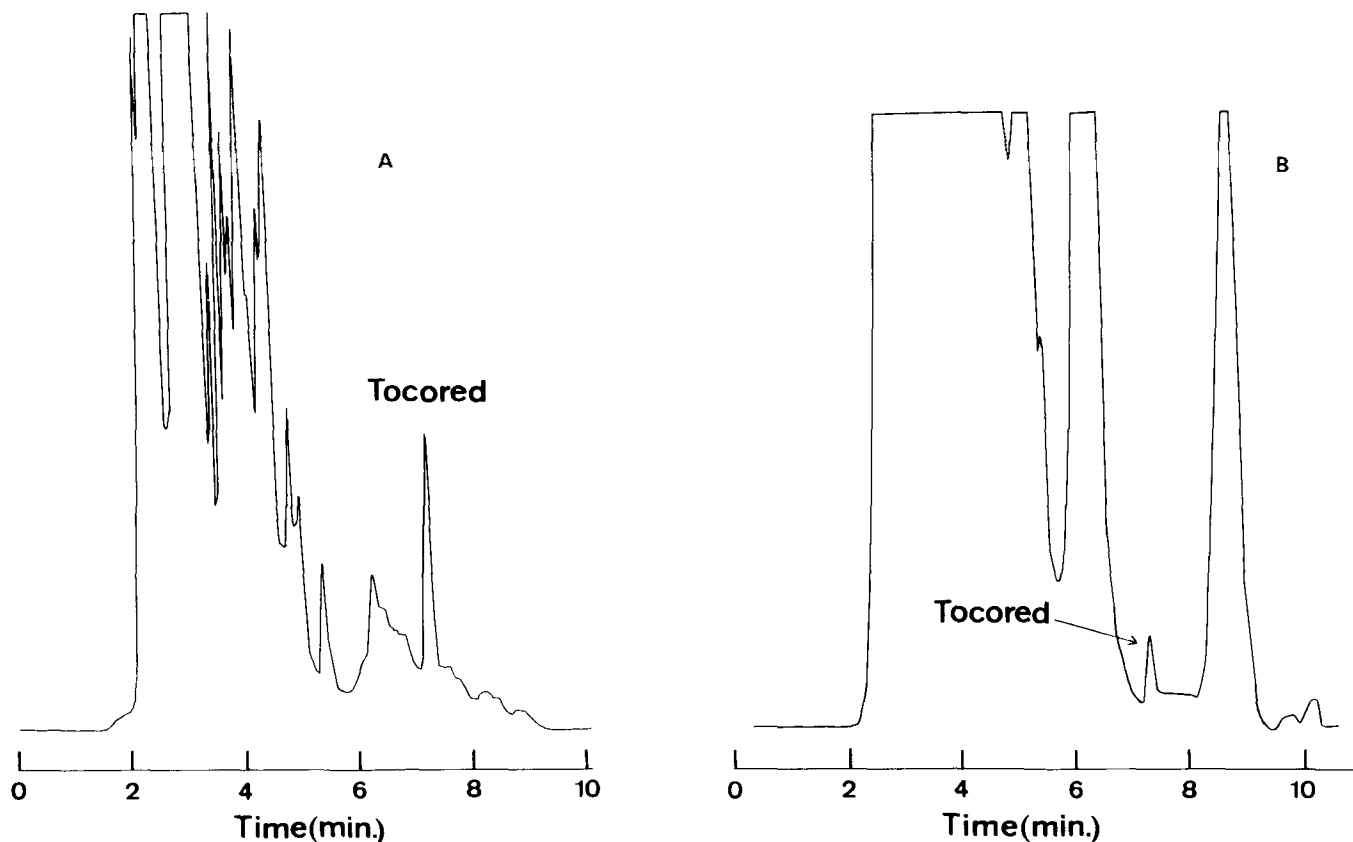


FIG. 4. HPLC chromatograms of tocopherol in soybean oil. A, crude and degummed oil; B, neutralized, bleached and deodorized oil.

from soybeans with different moisture contents at different refining stages, as shown in Table 4. These findings again indicated that  $\gamma$ -tocopherol is directly related to the color reversion.

Komoda et al. (6) also reported that more  $\gamma$ -TED was detected in the crude oil made from soybeans with higher moisture content. However, as shown in Table 4, our data indicated that in every step of refining, the oil sample generated from soybeans with higher moisture content always had less of both  $\gamma$ -tocopherol and  $\gamma$ -TED. As shown in Figure 2, the amounts of  $\gamma$ -tocopherol and  $\gamma$ -TED decreased only slightly during storage, while the color development reached its maximum level. Implicated in these results is that some other components might be present in deodorized oil to cause color reversion during storage. The  $\gamma$ -tocopherol and  $\gamma$ -TED are only the precursors of the direct causative agents.

With the higher moisture content in raw soybeans, the enzymes may be more reactive and the conversion rates of  $\gamma$ -Tocopherol to  $\gamma$ -TED and  $\gamma$ -TED to other oxidation products (such as tocopherol) will then be accelerated. Thus, the less  $\gamma$ -tocopherol and  $\gamma$ -TED left in oil, the more of the color causing species (or the precursors) should be formed, and consequently, the more color reversion in soybean salad oil should occur.

As described above,  $\gamma$ -tocopherol and  $\gamma$ -TED are the indirect precursors of the color reversion. Therefore, their contents in crude oil can be used as an index (or indices) for predicting color quality (i.e., color rever-

sion) of the deodorized oil. As shown in Figure 3, if crude oil contains more than 320 ppm of  $\gamma$ -tocopherol, or 160 ppm of  $\gamma$ -TED, or 480 ppm of both components in total, the soybean salad oil obtained after refining will meet the color requirement of NSPA (National Soybean Processing Association, U.S.A.).

The HPLC chromatograms of tocopherol in soybean oil are shown in Figure 4. The composition of soybean oil seemed to have great changes after the bleaching step. Some components might have been oxidized by active clay in the bleaching step (18-19).

Tocopherol is known to have antioxidative potency (20-21); therefore, it might be self-oxidized by  $O_2$  yielding some color reverted substances during storage of the soybean salad oil. There was a close relationship ( $r = 0.92$ ) between the tocopherol content in crude oil and the Rm value that the soybean salad oil reached during color reversion. As shown in Figure 5, the tocopherol content of soybean oil changed in different refining steps, especially in the process of neutralization. This suggested that tocopherol is not stable under alkaline conditions (18-19). Tocopherol content was further decreased in the bleaching step, which might be due to oxidation induced by active clay (20-21). However, the amount of tocopherol increased again after the heat treatment in the deodorizing step. It was then decreased gradually during color reversion in the soybean salad oil, as shown in Figure 6. This correlation evidently indicates that tocopherol is one of the proximate precursors causing the color reversion in soybean salad oil.

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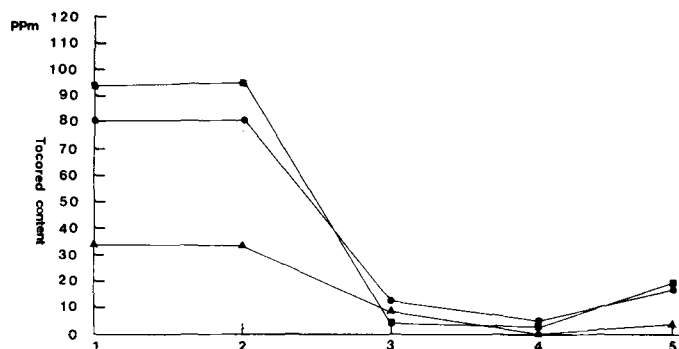


FIG. 5. Changes in tocored contents of soybean oils during refining process with various moisture contents of soybeans. ■, 18.2%; ●, 14.9%; ▲, 11.1%. 1, crude oil; 2, degummed oil; 3, neutralized oil; 4, bleached oil; 5, deodorized oil.

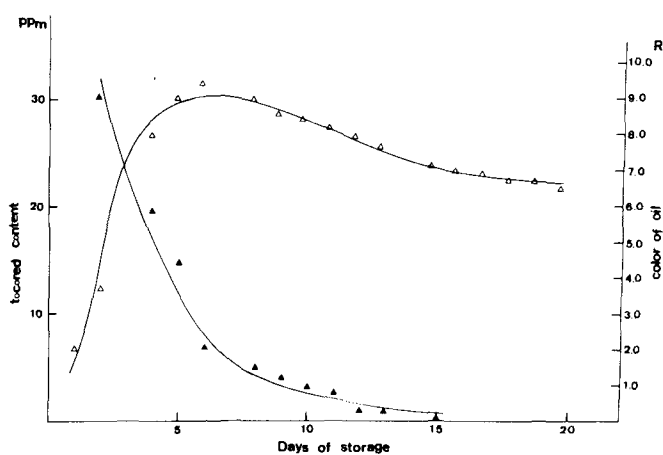


FIG. 6. Changes of tocored in soybean salad oil during color reversion. Δ, color; ▲, tocored.

Thus, the tocored content in initial deodorized oil is directly related ( $r = 0.98$ ) to the  $R_m$  reached during color reversion in that oil, but it was not so closely related ( $r = 0.84$ ) to the  $R_i$  as shown in Figure 7.

Although the initial deodorized oil contained more tocored than that of the color reverted oil, it was paler than the color reverted oil. Therefore, it might be concluded that there are other precursors in the initial deodorized oil (paler in color) that are converted to the color reverted substances (darker in color) during storage. This further supports our earlier statement that tocored is just one of the precursors of color reversion in soybean salad oil.

Careful examination of the HPLC chromatogram (Fig. 8) of the soybean salad oil led to the finding of a compound whose content was closely related ( $r = 0.95$ ) to the color of deodorized oil after storage, as shown in Figure 9. Because its structure was not known, it was conveniently labeled compound x. As shown in Figure 10, compound x was generated mainly from the precursors in neutralized oil in the process of bleaching, and its content in salad oil was shown to be related to the intensity of the red color developed in the color reversion. However, the amount of compound x in soybean salad oil did not change during storage. These results suggest that compound x is not the color-

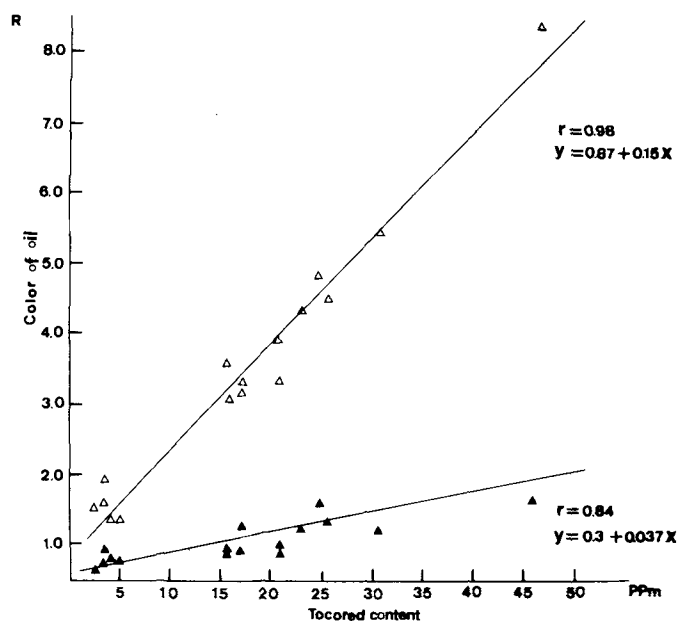


FIG. 7. Correlation of tocored contents in fresh deodorized oil with the  $R_i$  and  $R_m$  of soybean salad oil during color reversion. ▲,  $R_i$ ; Δ,  $R_m$ .

causing species, but the quantities of compound x and the color reverted substances have a linear relationship. The results mentioned above (Figs. 5 and 10) allow one to conclude that the conversion of precursors (involving tocored) was completed in the bleaching step, possibly via oxidation by active clay. Due to the existence of these color reverted substances, the color of bleached oil was very closely related ( $\gamma = 0.96$ ) to  $R_m$  reached during color reversion in corresponding soybean salad oil, as shown in Figure 11.

The color of the bleached oil was reduced significantly as the temperature reached  $230^\circ\text{C}$  during deodorizing. When the aged soybean salad oil was subjected to redeodorization, it was found that the redeodorized oil after a second cycle of color reversion gave an  $R_m$  value of magnitude similar to the  $R_m$  of the original aged salad oil before redeodorization. This fact indicated that the interconversion of the precursors and color reverted substances might be reversible, i.e.,

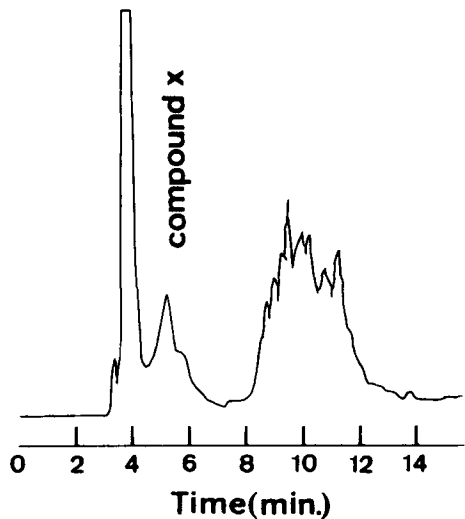


FIG. 8. HPLC chromatogram of compound x in soybean salad oil.

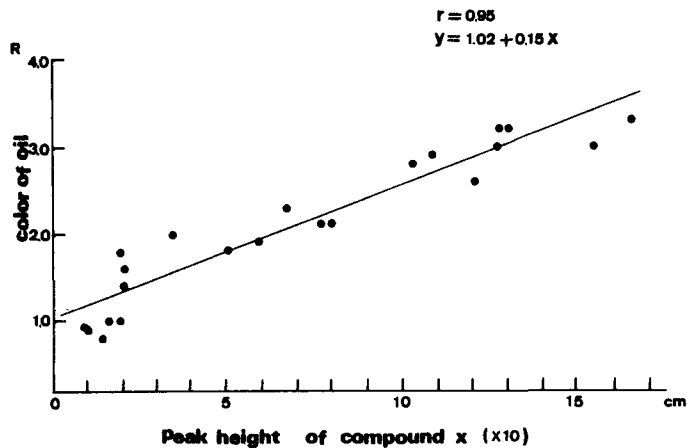


FIG. 9. Correlation of peak height of compound x in HPLC chromatogram with the red color in soybean oil.

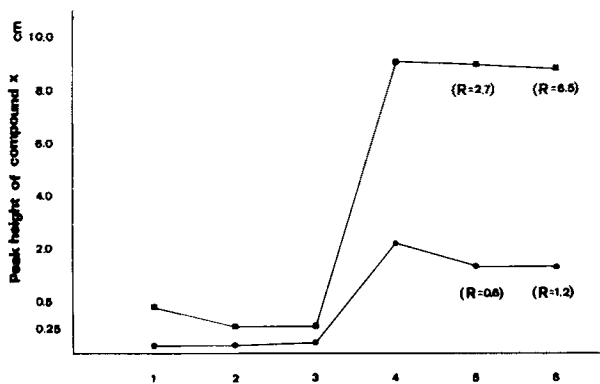


FIG. 10. Changes of the peak height of compound x in HPLC chromatogram during refining processes. 1, crude oil; 2, degummed oil; 3, neutralized oil; 4, bleached oil; 5, deodorized oil; 6, color reverted oil. ●, 10.7% moisture; ■, 16.4% moisture.

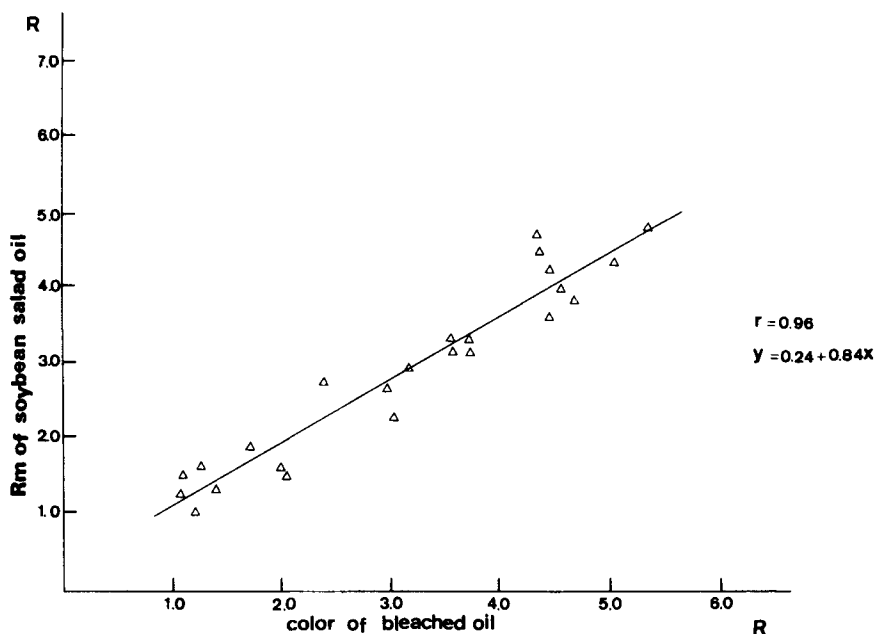


FIG. 11. Correlation of the color value of bleached oil with the Rm of soybean salad oil reached during storage.

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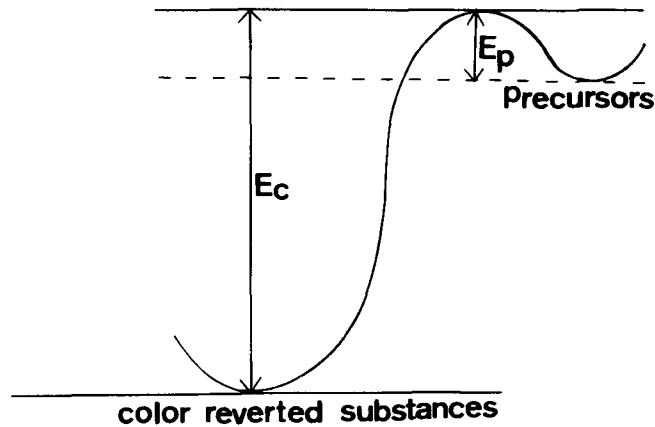
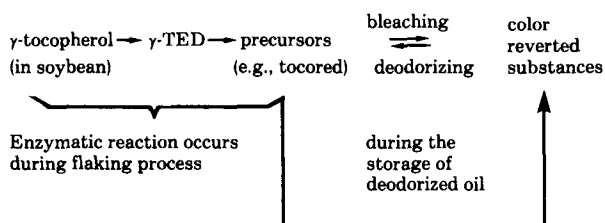


FIG. 12. Activation energy diagram of color reverted substances and its precursors.

the color reverted substances might be converted back to the original precursors at high temperature leading to reduction of color.

The above deodorization and subsequent color reversion phenomenon may be explainable with the aid of an activation energy diagram. As shown in Figure 12, the color reverted substances may be thermodynamically more stable than the precursors. The precursors could easily overcome the lower activation energy ( $E_p$ ) barrier and be converted into color reverted substances via oxidation by active clay in the bleaching step. Those color reverted substances might be converted back to the precursors by overcoming the higher activation energy barrier ( $E_c$ ) at elevated temperature during deodorization (deodorized at 235°C, 3-mm Hg abs.). The regeneration of these precursors allows a color reversion to occur.

From the results discussed in this report, the possible mechanism of color reversion in soybean salad oil could be concluded as follows:



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