

# Effect of Soybean Pretreatment on the Color Quality of Soybean Oil

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Color reversion in soybean oil can be prevented by reducing the enzyme activity of soybeans before cracking and flaking. Soybean oil extracted from steamed, intact soybeans (18% moisture) had lower R<sub>m</sub> (max. red) values in RBD oil, higher amounts of  $\gamma$ -tocopherol, plus its isomers, in both crude and RBD oil, and also higher amounts of hydratable phosphatides in crude oil than those in the oils from the same beans without steam treatment. For soybean pretreatments, a toasting process is less effective than the steaming process for the inhibition of color reversion of soybean oil. To prevent the occurrence of color reversion in RBD soybean oil, the amount of  $\gamma$ -tocopherol and  $\gamma$ -TED (5-[tocopheryloxy]- $\gamma$ -tocopherol) should be above 550 ppm in crude oil.

Soybean oil, after deodorization, is a pale light-colored product when freshly prepared. However, sometimes the oil color becomes darker during storage. This phenomenon is called color reversion, and has been a problem for the Taiwan oil industry which imports a large amount of soybeans. Komoda *et al.* (1) reported that the degree of color change depended on the nature of the raw soybeans, refining and deodorization conditions, duration of storage, etc. In this study, we found that the moisture content of soybeans was closely related to the oil quality and its color. Extensive studies of soybean oil quality have been made by several researchers. For example, List and Erickson (2) reviewed research indicating that excessive moisture of soybeans resulted in deteriorated soybean oil quality. Robertson *et al.* (3) found that the oil extracted from soybeans with high moisture content showed a corresponding increase in free fatty acids, Lovibond color and contents of oxidation deterioration products. As to color reversion, several reports (4,5) noted that higher moisture content in soybeans caused severe color reversion in RBD soybean oil. The compounds responsible for the color reversion were first reported by Swift *et al.* (6) who indicated that  $\gamma$ -tocopherol was the precursor of color reversion in deodorized cottonseed oil. Moreover, Lai *et al.* (7) recently illustrated the cause of color reversion in soybean oil. They found that  $\gamma$ -tocopherol and  $\gamma$ -TED (5-[tocopheryloxy]- $\gamma$ -tocopherol) were the precursors of a color-reverted substance and the conversion is mainly caused by enzyme action which occurred very rapidly after disruption of soybeans. Therefore the inhibition of enzyme activity in soybeans before bean breakage is very important for the prevention of color reversion in RBD soybean oil, especially for damaged soybeans with high moisture content. For enzyme inactivation, Rice *et al.* (8) also noted that steam heat treatment of whole soybeans prior to oil extraction was beneficial to the quality of both oil and meal. In this paper, the effects of soybean pretreatments before oil processing on the color quality of RBD oil during storage are described. These pretreatments performed on damaged beans might be a resolution for the oil industry which cannot easily control the quality of soybeans during long transport.

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## MATERIALS AND METHODS

**Materials.** Raw soybeans used were imported US soybeans, FGIS Grade No. 2 with moisture content of ca. 12%. Whole intact soybeans were selected from these beans. Tocopherol isomers ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  forms) were purchased from E. Merck. The  $\gamma$ -TED was donated by Yukihiro Ishilawa, Hiroshima Food Research Institute, Japan. All solvents were used in the HPLC analyses of LC grade including n-hexane, isopropanol, isopropyl ether and alcohol. The n-hexane used for extraction of soybean oil was of commercial grade.

**General methods.** Moisture content was determined by the AOCS method (9). Oil color was estimated by a Lovibond Automatic Tintometer. A one-inch cell was used to measure the crude and degummed oil, while a 5/8-inch cell was used in the measurements of neutralized, bleached and deodorized oil. The color reversion test was performed on the oil stored at room temperature. R<sub>m</sub> is defined as the highest red color value obtained during color reversion in RBD soybean oil.

**Raw soybean preparation.** Partially damaged soybeans were prepared by hydrating a 3 kg/batch to 18% moisture, which was stored in an airtight vessel and allowed to equilibrate for one week. These soybeans were shaken and aerated twice every day. After storage, soybeans were subjected to steaming or toasting pretreatment before oil extraction and processing. In the steaming process, soybeans were treated by live steam at 100°C for 1, 1.5 and 2 min, respectively. After steaming, these soybeans were then dried to 13% moisture content at 90°C for 20 min. In the toasting process, three different temperatures at 110, 130 and 150°C were selected to toast soybeans for 30 min, respectively. The soybeans treated by the steaming or toasting process were then processed according to the sequence of crushing, flaking and oil extraction. Desolventized crude oils were refined, bleached and deodorized to yield salad oil.

**Processing—extraction.** Soybean flakes were extracted using a Soxhlet extractor with n-hexane for three hr. The extracted oil was desolventized in a vacuum evaporator at 70°C. Gum was removed by adding 2% water (w/w) to the oil and then mixing at 60°C for 30 min before centrifugation.

**Refining.** The calculated amount of 18 Be' NaOH solution was added to the degummed oil which was preheated to 40°C and stirred for 10 min. The temperature was raised to 60°C and stirring continued for 5 min to complete saponification of free fatty acid before centrifugation. After separation of neutralized oil from the oil-soap mixture, the oil was washed with deionized water to remove soap completely.

**Bleaching.** Refined soybean oil was bleached using 1.5% activated clay (w/w). Bleaching was performed by heating the oil with vigorous stirring at 110°C under vacuum for 20 min and then cooling to 50°C followed by filtration.

**Deodorization.** Deodorization was accomplished in a laboratory scale by heating the oil under vacuum (3–5 mmHg) with sparging steam at 230–235°C for one hour and then cooling to 40°C to give a clean and clear oil.

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HPLC determination of tocopherol isomers,  $\gamma$ -TED and tocored. The tocopherol isomer,  $\gamma$ -TED and tocored content in crude and RBD soybean oil were determined by HPLC according to Lai *et al.* (7).

## RESULTS AND DISCUSSION

*Effect of the steaming process on the color reversion of soybean oil.* Previous reports (1,4,5,7) indicated that the moisture content of soybeans is closely related to the color reversion and amount of  $\gamma$ -tocopherol in soybean oil. Lai *et al.* (7) also indicated that the total amount of  $\gamma$ -tocopherol and  $\gamma$ -TED was inversely proportional to the Rm (maximum red) value of oil, i.e., the higher the moisture content in soybeans, the less amount of  $\gamma$ -tocopherol and more severe color reversion in the oil during storage. In this study, RBD soybean oil prepared from intact soybeans with 18% moisture content without any pretreatment (control oil) had quite low contents of  $\gamma$ -tocopherol and  $\gamma$ -TED, and the Rm value of oil reached 12 during storage (Table 1). However, if these soybeans were subjected to the steaming process before oil extraction, the resulting RBD soybean oils (oil 2S thru 4S in Table 1) had Rm values of only 1.3–1.4 during storage. In addition, all these oils contained a higher amount of  $\gamma$ -tocopherol but lower amounts of  $\gamma$ -TED and tocored than control oil (Table 2). The results in Table 1 and 2 also showed that the steaming of soybeans at 100°C for 1.5 min, was adequate to prevent the occurrence of color reversion in RBD soybean oil. The effects of various steaming processes on the red and Rm values, and the amount of  $\gamma$ -tocopherol or  $\gamma$ -tocopherol plus  $\gamma$ -TED in RBD soybean oil are illustrated in Figure 1. Both Rm and red value of RBD soybean oil from steamed, intact soybeans were inversely proportional to the amount of  $\gamma$ -tocopherol and the total amount of  $\gamma$ -tocopherol plus  $\gamma$ -TED in RBD soybean oil during color reversion. However, steam pretreatment of soybean flakes had little effect on the prevention of color reversion in RBD oil. As shown in Tables 1 and 2, the Rm value and the amount of  $\gamma$ -tocopherol of soybean oil, 6S obtained by steaming beans after flaking, were quite close to those of control

oil. The great decrease in the amount of  $\gamma$ -tocopherol for oil 6S indicated that the enzyme reaction for the conversion of  $\gamma$ -tocopherol into  $\gamma$ -TED, tocored or other oxidation products had proceeded before or during flaking. These results agreed with those reported by Lai *et al.* (7), who also suggested that the enzymatic conversion occurred during the flaking process. The high amount of  $\gamma$ -tocopherol for oil 2S through 4S indicated that the oxidation of  $\gamma$ -tocopherol into  $\gamma$ -TED or tocored through enzymatic reaction, was effectively inhibited by the steaming process. In order to completely inhibit color reversion of soybean oil, the process should be performed before bean disruption, because the reaction of the enzymes on the precursors of red substances occurred very rapidly once the beans were disrupted. Otherwise, the holding time of broken beans before being subjected to steaming should be as short as possible. Tables 1 and 2 showed the RBD oil of 5S from cracked beans still maintained low Rm value and a high amount of  $\gamma$ -tocopherol, because the broken soybeans were subjected to steaming immediately after the cracking process.

Steam treatment of bean flakes was not effective to prevent color reversion, i.e. oil 6S as shown in Table 1. This phenomenon might be explained by the fact that the moisture content at the particle surface of the ruptured tissue after cracking, increased during conditioning and was suitable for enzyme activity. Moreover, the next step, flaking, greatly exacerbates the problem by extensive rupture of the cells. Thus, the moisture content, contact with air, temperature and time were suitable for the enzyme reaction with  $\gamma$ -tocopherol,  $\gamma$ -TED or precursors of color reverted substance to produce a high amount of red compounds, resulting in the high Rm value of RBD soybean oil during storage. All these results indicated that color reversion of RBD soybean oil is caused by enzyme reaction which occurred very rapidly on broken beans or bean flakes.

To facilitate the process of oil extraction, soybeans should be dried to the desired moisture content after steaming. In the experiment, the moisture content of soybeans would rise from 12.88% to 16.4% after steaming at 100°C for 1.5 min. Drying at 90°C for 20 min was

TABLE 1

Effect of Soybean Pretreatment on the Color Quality of Soybean Oil

| Oil No. <sup>a</sup> | Condition                      | Oil color (R/Y)          |                         |                      |
|----------------------|--------------------------------|--------------------------|-------------------------|----------------------|
|                      |                                | Crude (1 <sup>''</sup> ) | RBD (5¼ <sup>''</sup> ) | Maximum <sup>b</sup> |
| 1C                   | control                        | 6.2/70 <sup>+</sup>      | 3.6/42                  | 12/70                |
| 2S                   | 100°C, 1 min                   | 7.2/46                   | 0.5/3.0                 | 1.4/10               |
| 3S                   | 100°C, 1.5 min                 | 6.7/40                   | 0.5/2.6                 | 1.3/9.4              |
| 4S                   | 100°C, 2.0 min                 | 6.0/37                   | 0.5/3.1                 | 1.4/10               |
| 5S                   | 100°C, 1.5 min (cracked beans) | 6.0/70 <sup>+</sup>      | 0.7/3.9                 | 1.5/11               |
| 6S                   | 100°C, 1.5 min (bean flakes)   | 7.6/54                   | 13/70 <sup>+</sup>      | 17/70 <sup>+</sup>   |
| 7T                   | 110°C, 30 min                  | 5.7/70 <sup>+</sup>      | 2.2/13                  | 4.2/35               |
| 8T                   | 130°C, 30 min                  | 5.5/70 <sup>+</sup>      | 1.4/6.7                 | 2.5/20               |
| 9T                   | 150°C, 30 min                  | 4.9/52                   | 1.2/7.6                 | 2.1/14               |

<sup>a</sup>All soybeans contain 18% moisture including control group; S stands for steaming and T stands for toasting; Oil No. 1C through 4S and 7T through 9T are prepared from intact soybeans.

<sup>b</sup>Maximum value (R/Y) of soybean oil during color reversion.

TABLE 2

Effect of Soybean Pretreatment on the Content of Tocopherol and Its Derivatives of Soybean Oil

| Oil No. <sup>a</sup> | Condition                      | $\gamma$ -Tocopherol (ppm) |     | $\gamma$ -TED (ppm) |     | Tocored (ppm) |    |
|----------------------|--------------------------------|----------------------------|-----|---------------------|-----|---------------|----|
|                      |                                | C                          | D   | C                   | D   | C             | D  |
| 1C                   | control                        | 92                         | 100 | 93                  | 60  | 3             | —  |
| 2S                   | 100°C, 1 min                   | 733                        | 632 | 30                  | 42  | 6             | 49 |
| 3S                   | 100°C, 1.5 min                 | 651                        | 679 | 28                  | 38  | 4             | 51 |
| 4S                   | 100°C, 2.0 min                 | 686                        | 671 | 28                  | 31  | 8             | 52 |
| 5S                   | 100°C, 1.5 min (cracked beans) | 621                        | 557 | 40                  | 51  | 13            | 67 |
| 6S                   | 100°C, 1.5 min (bean flakes)   | 106                        | 112 | 43                  | 48  | 20            | —  |
| 7T                   | 110°C, 30 min                  | 227                        | 253 | 114                 | 110 | 55            | 40 |
| 8T                   | 130°C, 30 min                  | 377                        | 347 | 88                  | 100 | 58            | 27 |
| 9T                   | 150°C, 30 min                  | 452                        | 421 | 59                  | 73  | 9             | 31 |

<sup>a</sup>According to Table 1.

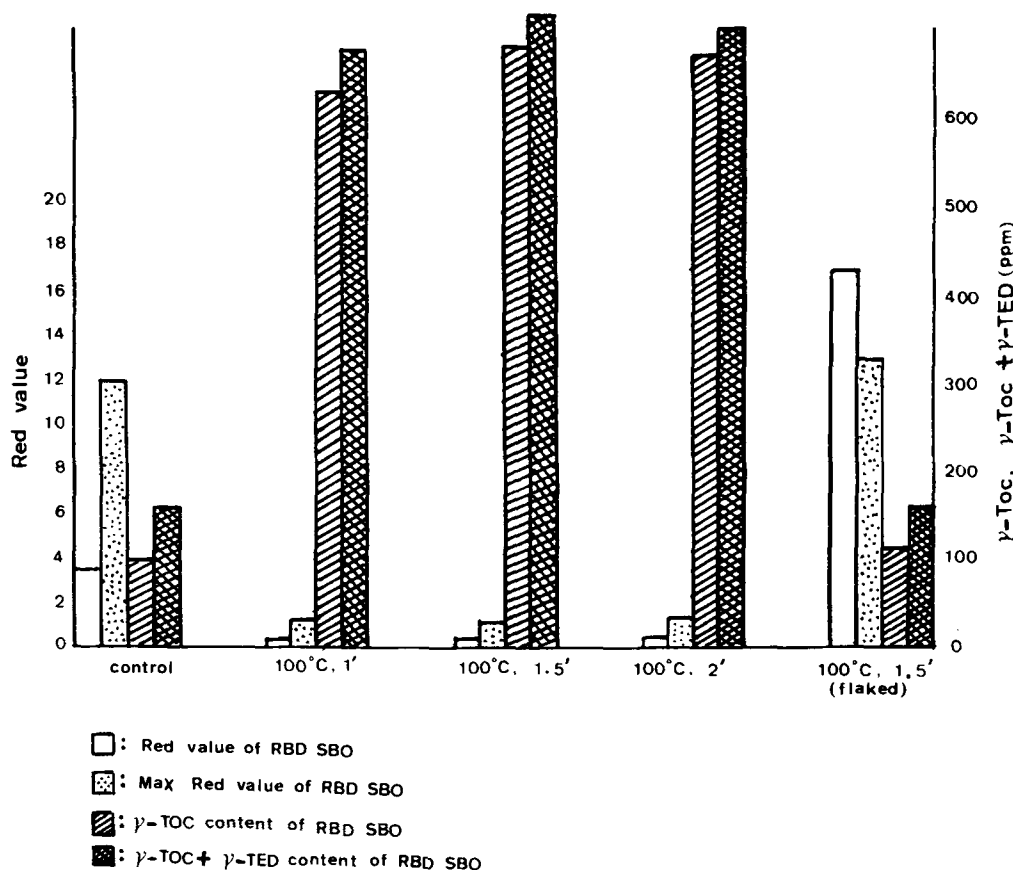


FIG. 1. Steam effects on the max. red values, the amount of  $\gamma$ -tocopherol and the total amount of  $\gamma$ -tocopherol plus  $\gamma$ -TED in RBD soybean oil.

required to reduce the moisture content of beans from 16.4% to 12.95%.

The amount of hydratable phosphatides in the crude oil from steamed beans (the difference in phosphorus content between crude and degummed oil) was higher than that from control beans. These hydratable phosphatides could be easily removed by water degumming (Table 3) and the resulting salad oil had the least degree of color reversion during storage.

*Effect of toasting on the color reversion of soybean oil.* The temperature for toasting intact soybeans before bean disruption had a significant effect on prevention of color reversion in soybean oil. The RBD oil from 18% moisture soybeans toasted at 150°C had the lowest Rm value of 2.1 compared to Rm values of 4.2 and 2.5 for the oils from beans toasted at 110°C and 130°C respectively (Table 1). In comparison to the steaming pretreatment, toasting is less effective for the inhibition of enzyme activity of soybeans. The residual enzyme activity in toasted soybeans led to the conversion of  $\gamma$ -tocopherol into  $\gamma$ -TED or the red compounds which finally remained in RBD soybean oil. The lower amount of  $\gamma$ -tocopherol in both crude and RBD oils (oil 7T through 9T, Table 2) from toasted soybeans again indicated that the enzymes responsible for the conversion of  $\gamma$ -tocopherol into  $\gamma$ -TED were not totally inhibited by the toasting process. The incomplete inhibition of  $\gamma$ -tocopherol conversion led to the formation of  $\gamma$ -TED and subsequently some red compounds which then

TABLE 3

Effect of Soybean Pretreatment on the Phosphorus Content of Soybean Oil

| Oil No. <sup>a</sup> | Condition                      | Phosphorus content (ppm) <sup>b</sup> |          |                          |
|----------------------|--------------------------------|---------------------------------------|----------|--------------------------|
|                      |                                | Crude                                 | Degummed | Reduction % <sup>c</sup> |
| 1C                   | control                        | 494                                   | 426      | 13.8                     |
| 2S                   | 100°C, 1 min                   | 708                                   | 40       | 94.4                     |
| 3S                   | 100°C, 1.5 min                 | 717                                   | 18       | 97.5                     |
| 4S                   | 100°C, 2 min                   | 563                                   | 34       | 94.0                     |
| 5S                   | 100°C, 1.5 min (cracked beans) | 606                                   | 22       | 96.4                     |
| 6S                   | 100°C, 1.5 min (bean flakes)   | 448                                   | 286      | 36.2                     |
| 7T                   | 110°C, 30 min                  | 535                                   | 187      | 65.0                     |
| 8T                   | 130°C, 30 min                  | 564                                   | 193      | 65.8                     |
| 9T                   | 150°C, 30 min                  | 587                                   | 208      | 64.5                     |

<sup>a</sup> According to Table 1.

<sup>b</sup> Equivalent phosphatides, ppm = phosphorus  $\times$  30.

<sup>c</sup> P reduction, % = P (crude) - P (degummed)/P (crude).

resulted in a different degree of color reversion of RBD soybean oil during storage. The data for the tocored analysis (Table 2) indicated that no correlation was found between the amount of tocored and the Rm value in RBD oil. This indicates that tocored was one of the precursors

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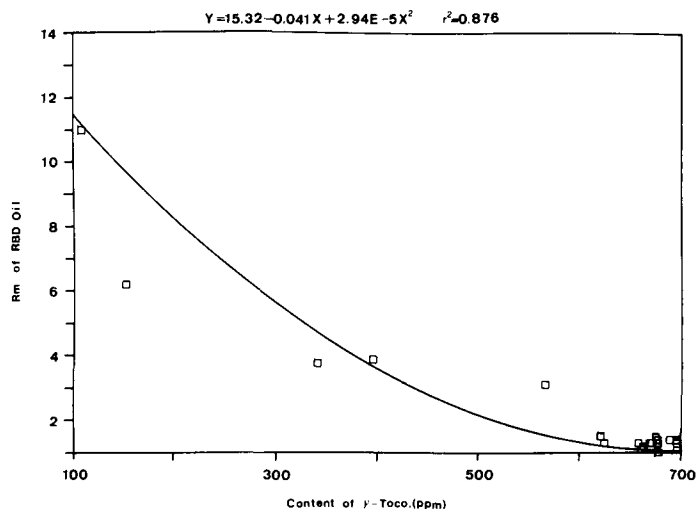


FIG. 2. Relationship between the amount of  $\gamma$ -tocopherol in crude soybean oil and the max. red value of RBD soybean oil during color reversion.

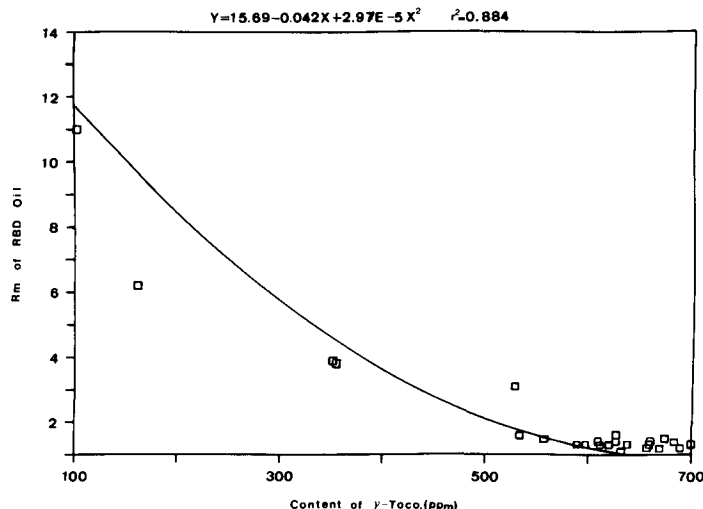


FIG. 4. Relationship between the amount of  $\gamma$ -tocopherol and the max. red value of RBD soybean oil during color reversion.

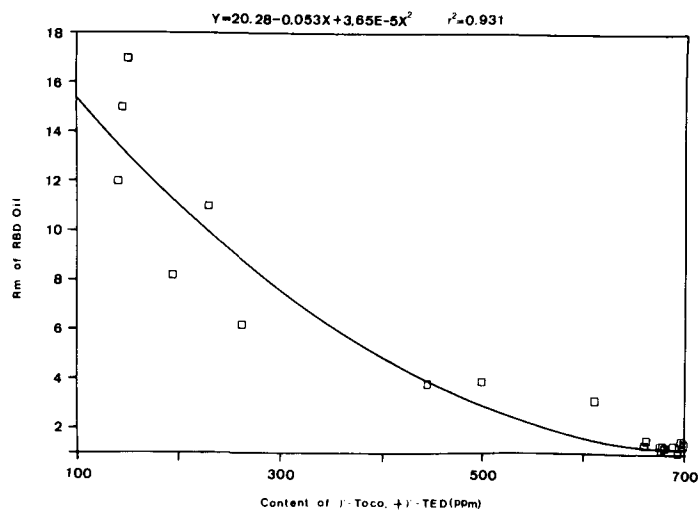


FIG. 3. Relationship between the total amount of  $\gamma$ -tocopherol and  $\gamma$ -TED in crude soybean oil and the max. red value of RBD soybean oil during color reversion.

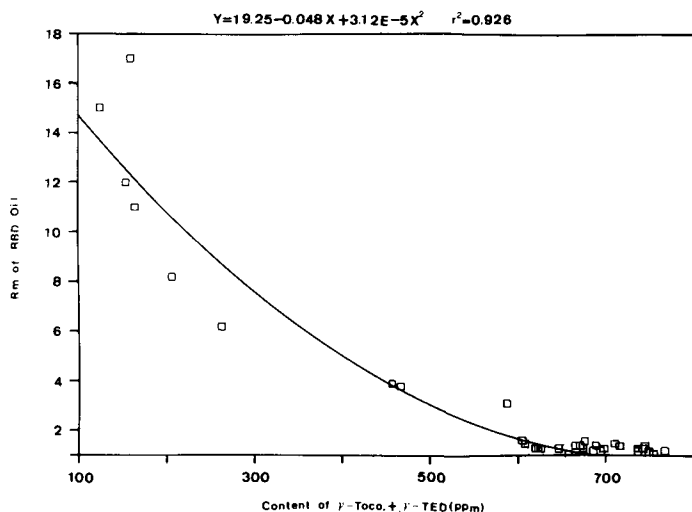


FIG. 5. Relationship between the total amount of  $\gamma$ -tocopherol plus  $\gamma$ -TED and the max. red value of RBD soybean oil during color reversion.

causing the color reversion in RBD soybean oil. The results for the analysis of phosphorus content (Table 3) also showed that toasting was less effective than steaming on the removal of phosphatides by water degumming.

*Correlation between the total amount of  $\gamma$ -tocopherol plus  $\gamma$ -TED and the max. red value in crude and RBD soybean oil during color reversion.* As shown in Figures 2 and 3, the amount of  $\gamma$ -tocopherol and the total amount of  $\gamma$ -tocopherol plus  $\gamma$ -TED in crude soybean oil ( $n = 36$ ) were inversely proportional to the Rm value of RBD soybean oil during color reversion in RBD oil. Also the amount of  $\gamma$ -tocopherol and the total amount of  $\gamma$ -tocopherol plus  $\gamma$ -TED in RBD soybean oil ( $n = 36$ ) were inversely proportional to the Rm value of RBD soybean oil during color reversion as shown in Figures 4 and 5. The occurrence of color reversion in RBD soybean oil can be predicted from the amount of  $\gamma$ -tocopherol or  $\gamma$ -tocopherol plus

$\gamma$ -TED in crude soybean oil. The curves in Figures 2 and 3 showed that the occurrence of color reversion in RBD soybean oil (the Rm value of RBD oil  $\leq 2.5$ ) could be prevented if the amount of  $\gamma$ -tocopherol or the total amount of  $\gamma$ -tocopherol plus  $\gamma$ -TED in crude oil was not less than 500 ppm and 550 ppm respectively. These values can be used as guidelines for the soybean oil processor to predict the color quality of RBD soybean oil.

*Correlation between the amount of hydratable phosphatides in crude oil and the degree of color reversion in RBD oil.* The removal of phosphatides by the degumming process affected the quality of finished oil. Kock and Penk reported that moist-heat treatment of soybean flakes prior to oil extraction would produce degummed soybean oil with very low phosphatides (9,10). In our study, we found that the amount of phosphatides in water-degummed oil from beans without pretreatment is

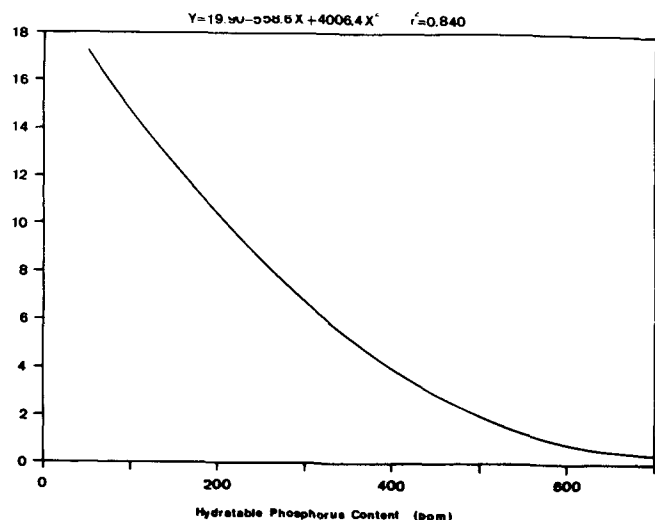


FIG. 6. Relationship between the amount of hydratable phosphorus in crude soybean oil and the max. red value of RBD soybean oil during color reversion.

much higher than that from steamed or toasted beans. Most of the phosphatides in crude oil from these two kinds of pretreated beans could be removed by water degumming as shown in Table 3. These results indicated that pretreatment of soybeans by a heating process greatly reduced the amount of non-hydratable phosphatides (NHP) in crude oil probably due to the inhibition of phospholipase activity and the resulting RBD oil had good color quality. However, the heating process should be conducted as quickly as possible on cracked or broken beans. Otherwise, the enzyme inactivation treatment on soybean flakes had no effect on the reduction of phosphatides (Table 3).

A positive correlation was found between Rm value of RBD soybean oil and the amount of hydratable phosphatides in crude soybean oil ( $n = 36$ ) (Figure 6). These results showed that the more hydratable the phosphatides in crude soybean oil, the less degree of color reversion in RBD soybean oil during storage. The data suggests that the degree of color reversion of soybean oil is greatly related to the quality of soybeans. Obviously, the amount of hydratable phosphatides in crude soybean oil can also

be used as an index to predict the degree of color reversion of RBD soybean oil during storage. For instance, when the amount of hydratable phosphatides in crude oil was above 500 ppm (Fig. 6), the Rm value of RBD soybean oil would remain below 1.5 during storage. In addition, based on the content of hydratable phosphatides in crude oil, preventive measures could be taken during refining, if the crude oil contained a high amount of hydratable phosphatides.

In order to keep good color quality of oil, soybean pretreatment is required especially if the soybeans are stored in an abused environment. Soybean pretreatment required would be reflected by the amount of hydratable phosphatides and  $\gamma$ -tocopherol in crude soybean oil.

#### ACKNOWLEDGMENT

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