Development of a Method for Chlorophyll Removal from Canola Oil Using Mineral Acids

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ABSTRACT: Because of the high level of chlorophyll-type compounds found in canola oil, bleaching is an important and critical step in the canola oil refining process. In this study, a new method for reducing the chlorophyll-type impurities prior to the bleaching step was developed. This method is based on precipitating the chlorophyll compounds with mineral acids. Concentrations of chlorophyll-type compounds of up to 30 ppm could be reduced to amounts of less than 0.01 ppm by mixing the crude canola oil with a 0.4 wt% mixture of phosphoric and sulfuric acids (2:0.75, vol/vol) for 5 min at 50°C. Centrifugation and filtration also were examined as two main methods for separating the chlorophyll precipitates. The results showed that filtration by a precoated textural filter with filter-aid clay could separate the precipitates as well as the centrifugation method.

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KEY WORDS: Bleaching, canola oil, chlorophyll, mineral acid.

Canola is one of the most important oilseed crops in the world. Research indicates that the FA composition of canola oil is especially favorable in terms of health benefits when used as part of a nutritionally balanced diet: It has a very low concentration of saturated fat (only 7%), is relatively high in monounsaturated fat, is among the highest in n-3 FA (10–11%), and contains a moderate level of polyunsaturated fat (32%). Canola oil is also a rich source of the antioxidant vitamin E. In addition, canoloa meal is an important source of protein in animal feeds (1–4).The pigments present in canola and other oilseeds are important factors, as they can impart an undesirable color to vegetable oils, promote oxidation in the presence of light, and inhibit the catalysts used for hydrogenation (5–7). The chlorophyll content in canola oil is significantly higher than those found in other vegetable oils and presents one of the biggest quality impediments for the canola oil industry (8). The removal of high levels of chlorophyll pigments from canola oil (50–60 ppm) requires large quantities of bleaching clays, resulting in high processing costs and significant losses of oil through adherence to the clays (9,10). Therefore, a cost-effective process for treating the oil extracted from canola seeds is needed to produce purified and degummed oil with low amounts of these chlorophyll-type compounds (9).

The main reason high levels of chlorophyll are present in rapeseed is that the seed freezes before it has significantly matured (9,11). Chlorophyll and chlorophyll-type compounds in the seed are mostly converted to pheophytin during the seed-conditioning step that occurs before oil extraction. Pheophytin is the main chlorophyll species present in crude and degummed oil (9). Because chlorophyll-type compounds are oil soluble, when the oil is extracted, it retains a green color, the intensity being proportional to the concentration of chlorophyll in the seed (12). Treating oil with mineral acids, such as phosphoric and sulfuric acids, cleaves the phytol group from the pheophytin molecules to form pheophorbide. The pheophorbide becomes protonated under highly acidic conditions and separates from the oil. Pheophorbide in its natural form is not water soluble, but treatment of its protonated form with a caustic, such as sodium hydroxide, causes the pheophorbide to be converted to sodium pheophorbide and become soluble in water (9). Thus, the pheophorbide can be separated from the oil before the neutralization step.

This paper examines several treatments of canola oil (containing up to 30 ppm chlorophyll-type compounds), with an emphasis on the process fundamentals of conventional refining required to reduce the level of chlorophyll-type impurities prior to the bleaching step. In this study, most of the operating parameters were optimized. As used herein, the term "chlorophyll-type compounds" refers to chlorophyll compounds measured as chlorophyll a by an automated PFX-990 Lovibond Tintometer.

MATERIALS AND METHODS

Materials. Rapeseed oils with chlorophyll contents ranging from 10 to 30 ppm were obtained from Behshahr Ind. Co. (Tehran, Iran). A bleaching earth, Tonsil Optimum 233, was purchased from Sue Chemie Co. (Jakarta, Indonesia). A filter-aid clay (Decalite clay) was obtained from Inorganic Ind. Co. (Tabriz, Iran). All chemical materials, including *p*-anisidine, acids, caustic solutions, reagents, and solvents, were of analytical grade and were obtained from Merck Co. (Darmstadt, Germany). TBHQ was purchased from Sinochem Tianjin Import & Export Corporation (Tianjin, China). Textural filters were obtained from Omid Co. (Tabriz, Iran).

Methods. Crude canola oil (500 g) and a mixture of sulfuric (98%) and phosphoric (85%) acids in ratios of 4:1 to 2:1 (vol/vol) at concentrations of 0.25 to 0.4 wt% were mixed with a mechanical stirrer at a speed of 200 rpm, at 50°C, for controlled times, which is also an important parameter for precipitating the chlorophyll-type compounds from the oil. The precipitates were

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*To whom correspondence should be addressed at Research and Development Laboratory of Savola Behshahr Ind. Co., 8th Km Fath Rd., Tehran, Iran. E-mail: e_sabbaghian@yahoo.com or Bahmaei@SBC.ir removed from the oil *via* centrifugation ($4000 \times g$, 5 min) or filtration by vacuum Büchner funnel (12 cm i.d.) through precoated textural filters by the filter-aid clay. To compare centrifugation with various filters, the acid-treated oil was filtered through the filter paper by gravity. The filtered oil was neutralized with 30% excess caustic solution (4 N) by a mechanical stirrer at 700 rpm and 80°C for 1 min and washed with hot water (20 wt%) to remove the soap created. It was then dried under vacuum to reduce the moisture of the oil to less than 0.1 wt%. Finally, based on the amount of chlorophyll in the dried oil, it was bleached with 0.8 to 1 wt% bleaching earth under vacuum at a temperature of 105–110°C for 30 min and filtered through Whatman no. 91 filter paper to separate the clays from the oil. The bleached oil was then deodorized at 250–255°C under 20 mmHg vacuum, with the help of dry ice, for 30 min with steam stripping. During these steps, parameters such as the chlorophyll content, acidity, color, and *p*-anisidine value of the oil were measured. The oil stability index (OSI) of the final samples was determined, and TBHQ was used as an antioxidant at a 100-ppm concentration.

Color and chlorophyll content. In each step of the procedure, the red color and chlorophyll content of the oil were measured with an automated PFX-990 Lovibond Tintometer.

Acidity. FFA were determined according to AOCS Official Method Ca 5a-40 (13).

Phosphatides. The phosphorus content of the final oil was determined by spectrophotometric measurement with a CE 2011 Cecil spectrophotometer according to AOCS Official Method Ca 12-55 (14).

p-Anisidine value. The *p*-anisidine value of the deodorized oil was determined according to AOCS Official Method Cd 18-90 (15) with a CE 2011 Cecil spectrophotometer.

OSI. OSI was measured after the deodorization step with a 679 Metrohm Rancimat instrument according to standard ISO 6886 (Draft International Standards, 1996).

Statistical analysis. All results are the means of three replications ± SD.

RESULTS AND DISCUSSION

Acid-mixture selection. As mentioned, mineral acids can precipitate chlorophyll-type compounds from the oils. The most popular mineral acids are phosphoric, sulfuric, nitric, and hydrochloric acids. Because of the highly corrosive properties of hydrochloric acid on metals, alloys, and even stainless steel, it is not suitable for use in the oil industry. Thus, phosphoric acid is usually used to remove phospholipid compounds in oils and fats processing. Adding other mineral acids increases its ability to precipitate chlorophyll-type compounds by decreasing the moisture content and protonation of the chlorophyll. Therefore, only three acids were investigated, phosphoric, sulfuric, and nitric. Mixtures (0.4 wt%) of phosphoric/sulfuric and phosphoric/nitric acids at a ratio of 2:1 (vol/vol) were added to crude canola oil at 50°C and were compared with phosphoric acid, which is added in the conventional methods. After 15 min, the precipitates were separated *via* centrifugation, and the procedure was completed as described in the Materials and Methods section. As illustrated in Figure 1, a mixture of phosphoric and sulfuric acids gave the best results under the same conditions.

Effect of the acid-mixture concentration. The concentration of the acid mixture is an important parameter in the chlorophyll degradation process. Increasing the amount of added acid increases chlorophyll precipitation but also increases the loss of oil (data not shown). Because of these effects, the optimal amount of added acid must be determined. Thus, in the first

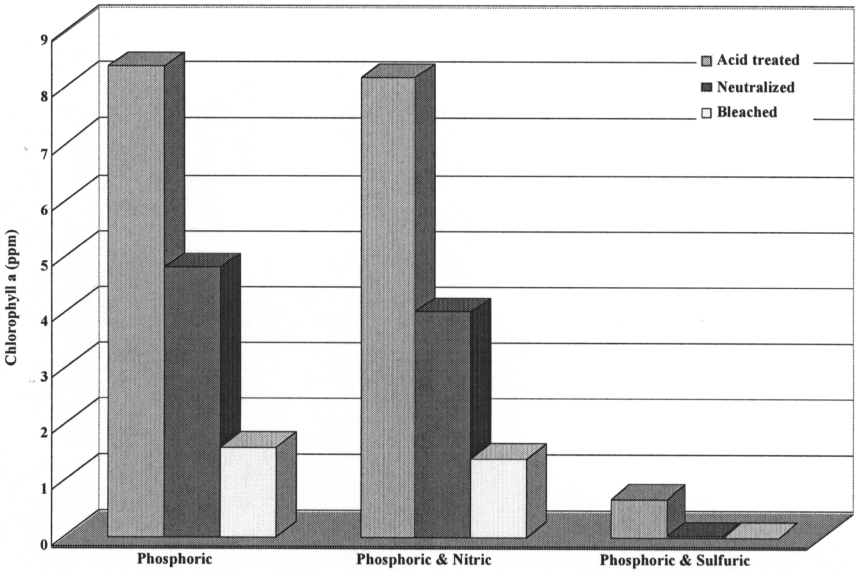

FIG. 1. Effect of different acid mixtures on the removal of chlorophyll from canola oil. Initial chlorophyll a content: 20 ppm; acid mixture: 0.4 wt% at a ratio of 2:1 (vol/vol); contact time of oil and acid mixture: 20 min at 50°C; bleaching earth: 1 wt%.

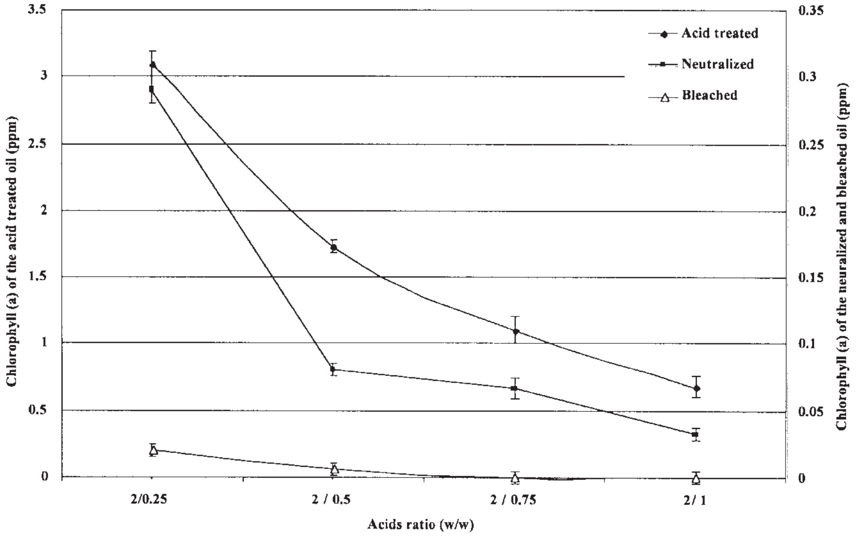

FIG. 2. Variations in the chlorophyll content with different acid-mixture ratios. Initial chlorophyll a content: 20 ppm; acid mixture: 0.4 wt% phosphoric/sulfuric acid mixture; contact time of oil and acid mixture: 20 min at 50°C; bleaching earth: 1 wt%.

step, we investigated the effect of the acid ratio. Different ratios of phosphoric/sulfuric acid mixtures were added in amounts of 0.4 wt% to crude canola oil at 50°C for the treatment, as described in the Materials and Methods section. According to Figure 2, as the ratio of phosphoric acid to sulfuric acid decreased, chlorophyll removal increased, and the best results were obtained with acid mixtures at ratios of less than 2:0.5 (vol/vol). The acid mixture at 2:0.25 (vol/vol) provided good results for low levels of chlorophyll. However, for the crude canola oil with a high concentration of chlorophyll-type compounds, using the higher acid-mixture ratio and amounts gave better results. In the second step, the phosphoric/sulfuric acid mixture at a ratio of 2:0.75 (vol/vol) was added to crude canola oil at concentrations of 0–0.8 wt% at 50°C to find suitable acid concentrations. After 15 min, the precipitates were removed and the procedure was completed as described in the Materials and Methods section. The results showed that acid mixtures in amounts higher than 0.4 wt% were the most effective in removing chlorophyll-type compounds (Fig. 3).

Effect of contact time. The particle size of the precipitates is important for good separation, which is directly related to the contact time between the acid mixture and the oil. Under the same conditions (reaction batch size and type of mixing), the particle size of the precipitates will increase with longer contact times, but the energy consumption and loss of oil will also increase. On the other hand, short contact times are not effective for chlorophyll removal. To investigate this important parameter, a 0.4 wt% mixture of phosphoric/sulfuric acids at a ratio of 2:0.75 (vol/vol) was added to crude canola oil at 50°C, and samples were withdrawn from the reaction mixture at regular time intervals. As shown in Figure 4, the protonation of chlorophyll-type compounds was a very fast reaction, and pheophorbide precipitates started to form in a few seconds. However, contact times over 2 min resulted in larger particles.

Separation of the precipitates. Centrifugation and filtration are the two main methods used to separate precipitates, and these two methods were examined in this study. The effectiveness of various filters, such as Whatman no. 41 and 42 GF/A filter paper, polishing filter paper, and precoated textural filters, were tested by passing the acid-treated oil through them. The filtered oil was centrifuged at a speed of 4000 × *g* for 5 min, and the chlorophyll contents of the filtered oils were measured and compared. As shown in Figure 5, centrifugation and filtration by precoated textural filters with filter-aid clay gave the same results, and these were better than other treatments. Because of the small ratio of precipitates to oil phases and oil-loss problems, the centrifugation method is unsuitable on an industrial scale. Therefore, we recommend the filtration method for separating chlorophyll-type precipitates on a large scale.

It should be noted the precoated textural filters can be reused several times (Fig. 6). Yet according to these data, the ability of the precoated textural filters to separate precipitated chlorophyll-type compounds decreased over time.

Effect of acid mixtures on acidity. Sulfuric acid can hydrolyze TG to FFA, and not using it in appropriate amounts causes an increase in the acidity of the oil and thus an increase in oil loss. To compare this method with conventional ones, two samples were prepared and tested. The first sample was treated with a 0.4 wt% mixture of phosphoric/sulfuric acids at a ratio of 2:0.75 (vol/vol), and the second sample was treated with 0.1 wt% phosphoric acid. The acidity of the two samples was measured before and after the separation of precipitates. For the sample treated with a 0.4 wt% mixture of phosphoric/sulfuric acids at a ratio of 2:0.75 (vol/vol), the results were as follows: before precipitate separation, 3.36 ± 0.27; after precipitate separation, 2.7 ± 0.13. For the sample treated with 0.1 wt% phos-

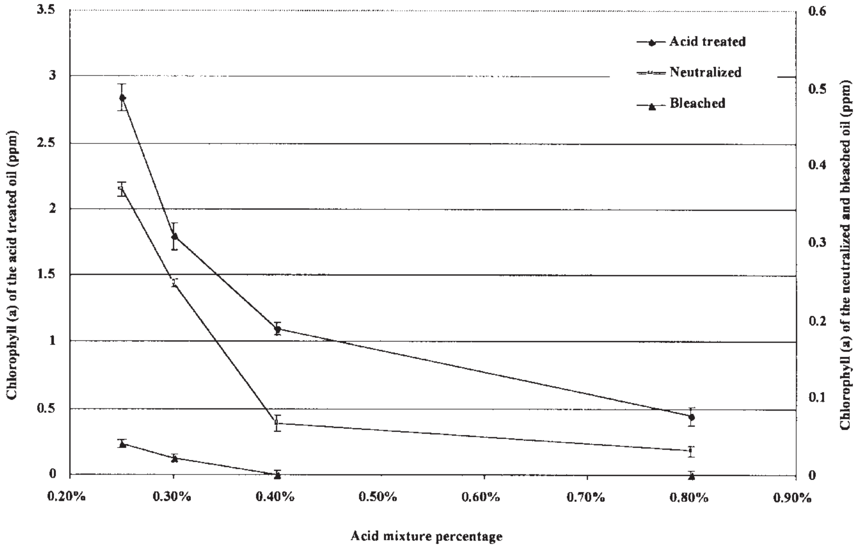

FIG. 3. Variations in the chlorophyll content with acid-mixture percentage after the bleaching step. Initial chlorophyll a content: 20 ppm; acid mixture: phosphoric/sulfuric acid mixture at a ratio of 2:1 (vol/vol); contact time of oil and acid mixture: 20 min at 50°C; bleaching earth: 1 wt%.

phoric acid, the results were as follows: before precipitate separation, 2.78 ± 0.11; after precipitate separation, 2.64 ± 0.12. There was no significant difference in acidity between the two samples. In addition, the acid-treated oil was neutralized with an excess amount of caustic solution a short time after the acid-treating step, thus minimizing the risk of higher losses due to acid hydrolysis of the TG.

Effect of acid mixtures on the red color. Compared with the conventional method (use of only phosphoric acid), use of the sulfuric/phosphoric acid mixture considerably increased the red color of the oil after bleaching, but the final deodorized oil had only a slightly red color after deodorization (Fig. 7). This can be related to a reversible interaction between the sulfuric acid and red color pigments in the acid-treatment step, which needs more investigation.

Stability of the oil. The OSI of the final refined, bleached,

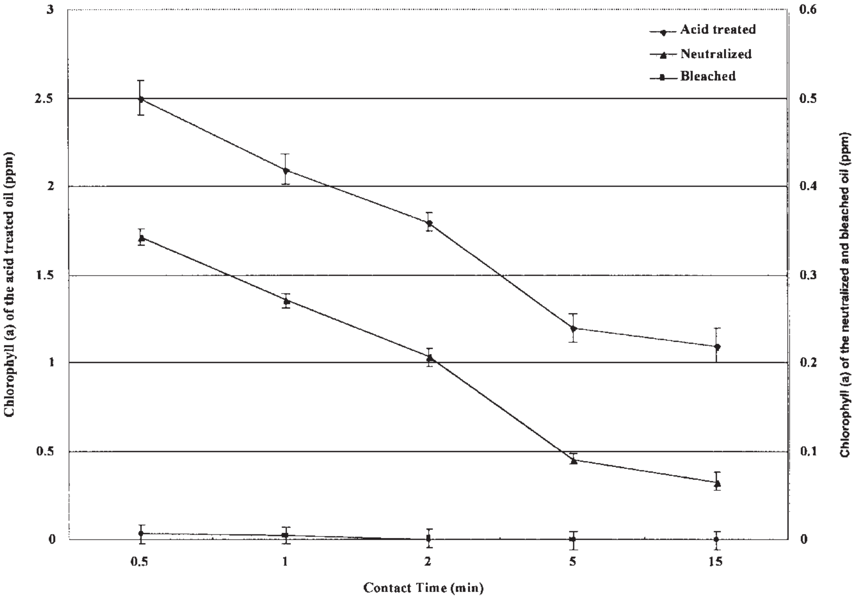

FIG. 4. Effect of contact time of the oil and acid mixture on chlorophyll degradation. Initial chlorophyll a content: 20 ppm; acid mixture: 0.4 wt% phosphoric/sulfuric acid mixture at a ratio of 2:0.75 (vol/vol); temperature: 50°C; bleaching earth: 1 wt%.

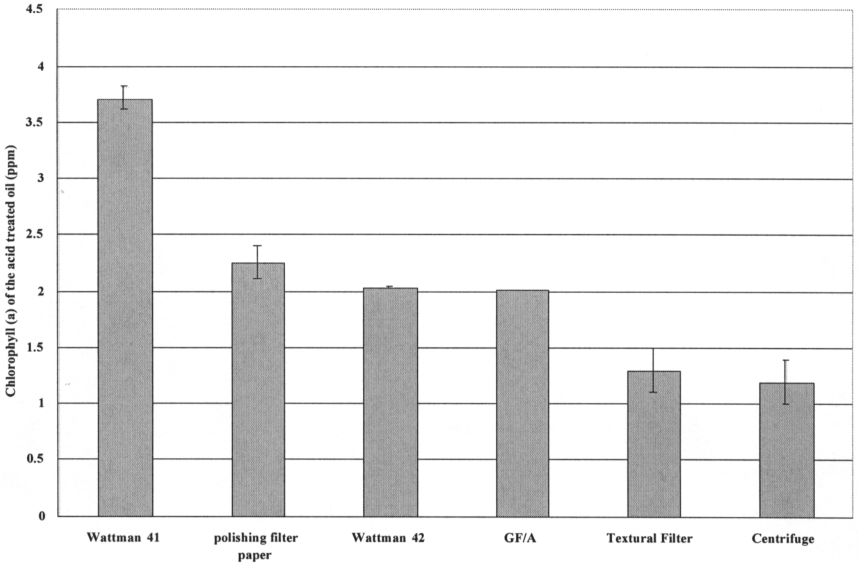

FIG. 5. Comparison of different filter papers with centrifugation for the separation of chlorophyll-type precipitates. Initial chlorophyll a content: 10 ppm; acid mixture: 0.4 wt% phosphoric/sulfuric acid mixture at a ratio of 2:0.75 (vol/vol); contact time of oil and acid mixture: 5 min at 50°C.

and deodorized oil sample produced by this method were 20 h and 9 h at 110°C in the presence and absence of TBHQ (100 ppm) as an antioxidant, respectively, whereas the OSI of oil processed using the conventional method were only 18 h and 8 h in the presence and absence of TBHQ, respectively. Also, the *p*-anisidine value of the oil after deodorizing was 5.34, which is better than the 6 or higher values of conventionally processed oil. The percentage of phosphatides in the final oil was less than 0.005%, the same as oil processed using the conventional method.

The concentration of chlorophyll-type compounds of up to 30 ppm could be reduced to amounts of less than 0.01 ppm in canola oil with a 0.4 wt% mixture of phosphoric/sulfuric acids at a ratio of 2:0.75 (vol/vol) and a contact time of 5 min at 50°C. Bleaching earth consumption decreased with an increase in OSI time. Thus, this method is more efficient than the conventional

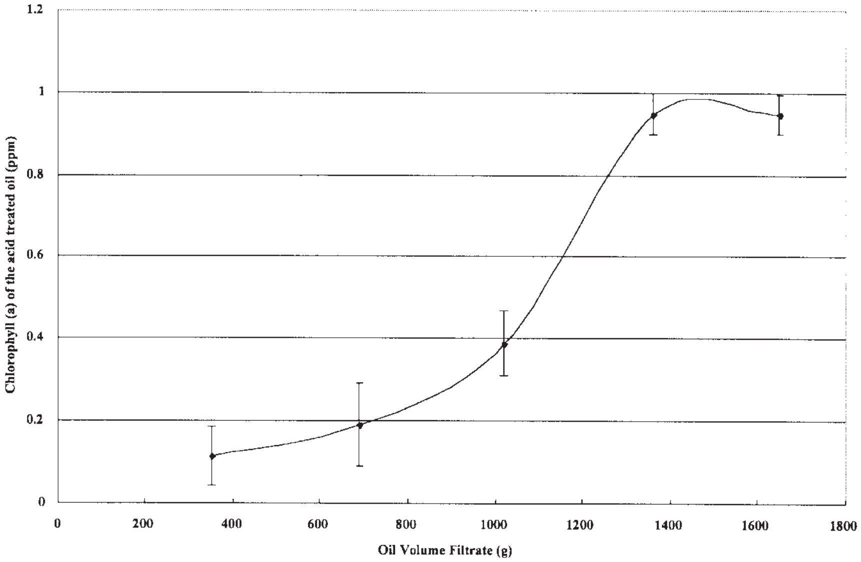

FIG. 6. Reusability of the precoated textural filter with filter-aid clay for chlorophyll precipitate separation. Initial chlorophyll a content: 10 ppm; acid mixture: 0.4 wt% phosphoric/sulfuric acid mixture at a ratio of 2:0.75 (vol/vol); contact time of oil and acid mixture: 5 min at 50°C. Filtration was done by a vacuum Büchner funnel with a 12-cm i.d.

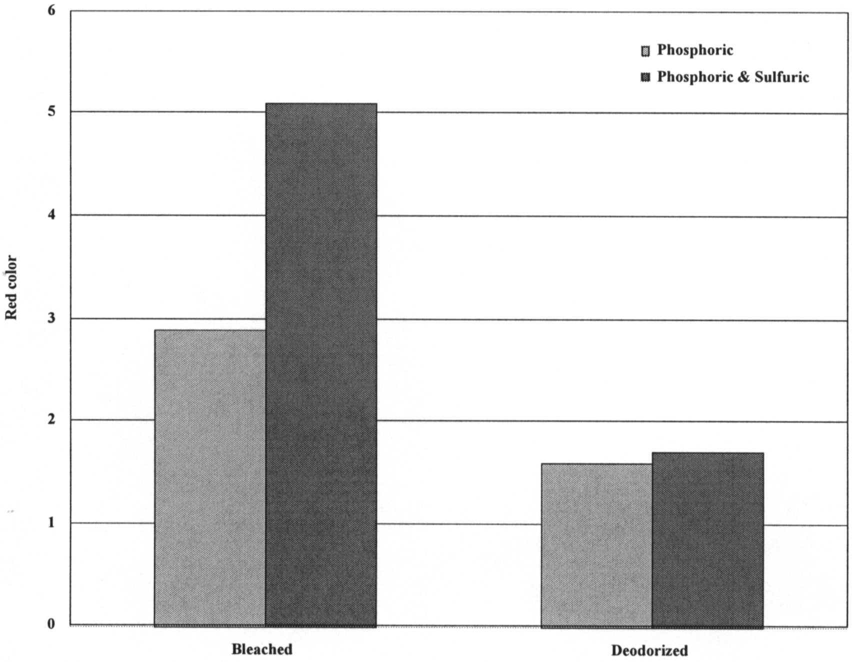

FIG. 7. Effect of the acid mixture on the red color of the oil. Initial chlorophyll a content: 10 ppm; acid mixture: 0.4 wt% phosphoric/sulfuric acid mixture at a ratio of 2:0.75 (vol/vol) or 0.1 wt% phosphoric acid; contact time of the oil and acid mixture: 5 min at 50°C.

method. The concentration of acid mixture and the amount of bleaching earth could be reduced to less than 0.3 wt% at a ratio of 2:0.5 (vol/vol), and to 0.75 wt%, respectively, when the concentration of chlorophyll was less than 20 ppm.

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