Possible Causes for Decreased Stability of Canola Oil Processed from Green Seed

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Canola oil extracted from seeds with a high-chlorophyll content can contain chlorophyll derivatives in excess of 30 ppm. When processed, this oil has been observed to be less stable than oil (typically containing 5 to 25 ppm chlorophyll) processed from high-grade seed. Possible causes for this phenomenon were investigated in this study. The effect of initial pheophytin content was examined by mixing fully saturated oil (tricapryloylglycerol) with increasing amounts of pheophytin and then by subjecting the mixtures to processing conditions. When the processed oils were combined with an unsaturated oil (canola oil), the oxidative stabilities decreased as the pre-processing content of pheophytin increased. Examination of the effect of increased bleaching to remove excessive levels of pheophytin showed that oil stability decreased with increasing exposure to bleaching clay. Additionally, processing treatments did not remove secondary autoxidation products from oil that was abused prior to processing. Such a finding revealed the importance of initial oil quality on processed oil stability, i.e., the greater abuse of the crude oil (resulting in greater contents of secondary oxidation products), the lower the stability of the processed oil. Finally, previous reports by other researchers of pheophytin's prooxidative effect in oil stored in light were confirmed.

KEY WORDS: Bleaching, canola oil, chlorophyll, stability, vegetable oil.

Crude canola oil is unique among the major edible oils in possessing a relatively high initial chlorophyll content, typically ranging from 5 to 25 ppm (1). High initial levels are decreased by modern methods of refining to levels required for consumer acceptance [less than 0.1 ppm (1)]. However, field conditions, such as frost damage, arrest the processes leading to clearing of chlorophyll that occurs in the final maturation stages of the seed (2). The resultant high-chlorophyll content is then transferred to the extracted oil. Modern processing methods can reduce this amount to a desirable level of less than 0.1 ppm; however, the fully processed oil is lower in stability than oil processed from high-quality undamaged seed (3).

Higher initial chlorophyll levels could readily give rise to at least three possible consequences that could each contribute to processed oil instability, depending upon the storage and processing of the high-chlorophyll oil. First, chlorophyll and its derivatives are powerful prooxidants under conditions of exposure to light (4,5). Commercial processes reduce these levels to less than 0.1 ppm (1); therefore, these compounds are probably not important contributors to lower quality in the processed oil (6). On the other hand, exposure of the unbleached oil to conditions favorable for the prooxidative effect of chlorophyll would produce oxidation products that could survive processing and could have a negative effect on the processed oil stability (7). Second, bleaching can have a deleterious effect on the stability of a vegetable oil (8), and more extensive bleaching (including extended exposure time and/or increased amounts of clay) required by an oil with a high content of chlorophyll derivatives may exacerbate this effect. Finally, processing conditions could produce new compounds from chlorophyll derivatives in the crude oil. Stability would be compromised if the compounds were prooxidant in nature.

The purpose of this study was to investigate the potential that these effects have for decreasing the stability of processed oil with a high initial content of chlorophyll derivatives.

MATERIALS AND METHODS

Tricapryloylglycerol (95% pure) and chlorophyll a (99% pure) were obtained from Sigma Chemical Co. (St. Louis, MO). The tricapryloylglycerol and fully processed canola and linseed oils (purchased commercially) were further purified (to remove any nontriacylglycerol components that may have interfered with subsequent autoxidation studies) *via* column chromatography on silica gel (70–230 mesh; BDH Inc., Edmonton, Alberta, Canada) in a 2:1 (w/w) ratio silica gel/oil in a 2.5-cm diameter column, with hexane as the eluting solvent. Oil purity was verified by thin-layer chromatography on aluminum-backed silica-gel plates (E.M. Separation, Gibbstown, NJ) with a mixture of hexane/diethyl ether/acetic acid (50:50:0.5, vol/vol/vol) as developing solvent. Visualization was accomplished by application of 2,7-dichlorofluorescein, followed by viewing under ultraviolet light, as well as exposure of the developed plates to iodine vapor.

Preparation and addition of pigments to oil. Pheophytin a was prepared by dissolving 1 mg chlorophyll a in 1 mL acetone and acidifying with 13% HCl. Hexane (1 mL) was added, followed by 1 mL deionized water. The nonpolar layer containing the pheophytin was removed and washed five more times with 1 mL deionized water, then added to a known weight of oil. A control was prepared with the same steps of said addition, hexane extraction and water wash as applied to 1 mL acetone with no added chlorophyll (hereafter referred to as a blank pheophytin preparation).

Processing conditions. All processing steps were carried out in controlled-temperature oil baths on IKAMAG RET-G stirrer/heaters (Rose Scientific Ltd., Edmonton, Alberta, Canada) and stirred at a rapid rotation rate.

Degumming was carried out at 40°C with a 15-min exposure of the oil to 0.2% (vol/wt) citric acid (50% wt/vol in water), followed by the addition of 2% (vol/wt) deionized water. After a further period of 15 min, the mixture was centrifuged at 4400 × *g* for 5 min, and the oil layer was removed by pipetting.

Refining involved an initial 1-h pretreatment at 40°C with 0.015% (vol/wt) of orthophosphoric acid. Sodium hydroxide (9.5% wt/vol) was added in sufficient volume to neutralize 1.1% free fatty acid. This amount represented the high end of typical values in the processing industry (1) and was not based on the content of free fatty acids in the experimental oil because the latter was a purified product and, as such, was not expected to contain any free

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fatty acids. After a 15-min exposure at 40°C, the mixture was centrifuged at 4400 × *g* for 5 min, and the oil layer was removed by pipette. The mixture was washed two more times with 15 mL deionized water at 70°C.

Unless otherwise stated, bleaching was accomplished with 5% (w/w) Filtrol 105 clay (Engelhard Corp., Jackson MS). The mixture was stirred for 30 min at 104°C under a continuous stream of nitrogen. The oil was centrifuged at 4400 × *g* for 15 min, to remove the clay, and filtered through a 0.2-μm Corning syringe filter (Baxter Canlab, Winnipeg, Manitoba, Canada). The absorbance between 600 to 700 nm of all oils that had pigment added to them prior to processing was determined (neat) to ascertain the extent of bleaching. If necessary, both the control oil and the treated oil(s) were re-bleached until no absorbance was seen in this region (indicating less than 65 ppb pheophytin a present).

Absorbance measurements. All measurements were made in a Milton Roy Spectronic model 1201 spectrophotometer (Rochester, NY) on aliquots of the oils solubilized in hexane.

Stability assessment. Aliquots of oil (0.1 g) were distributed in a series of 13 × 100-mm test tubes and placed in a forced-air oven at 60°C. At appropriate time intervals, two test tubes from each treatment were removed, and the absorbance at 234 nm was determined as indicative of conjugated diene content (9). The absorbances obtained were averaged, and the standard deviations were calculated for the duplicates of each treatment.

When tricapryloylglycerol was used as the carrier for the pheophytin during processing, the effect of any breakdown products was determined by thoroughly mixing equivalent weights of the tricapryloylglycerol and a purified canola or linseed oil, followed by stability assessment.

Assays. Free fatty acid content, anisidine and peroxide values were determined by the methods of Lowry and Tinsley (10), IUPAC (2.504) (11) and Takagi *et al.* (12), respectively. Iron and phosphorus contents were determined *via* inductively coupled plasma spectroscopy (ICP) by POS Pilot Plant Corp. [Saskatoon, Saskatchewan, Canada (13)]. Tocopherol analyses were performed by high-performance liquid chromatography (HPLC) by the method of Carpenter (14) on a Waters model 625 HPLC equipped with a Waters model 484 ultraviolet (UV) detector (Waters Chromatography, Milford, MA). Standards were used to determine the response factors of α-, β-, γ- and δ-tocopherols (Sigma Chemical Co.), and the amounts of these compounds detected in each oil were totalled and expressed as tocopherol content.

Statistical analysis. Analysis of covariance on the obtained slopes was performed when curves with straight-line portions contained sufficient data points. Otherwise, an analysis was performed within each experiment on data that were collected at the last time period prior to where the breakdown of primary oxidation products exceeded production (as evidenced by a leveling off of the curve for one or more samples). This point represented the optimum for analysis as it included both the effects of the induction period length, as well as the rate of build-up of primary oxidation products. Significance was expressed as the 0.05 level of probability. A Newman-Keul's multiple range test was used to determine statistical significance between samples.

EXPERIMENTAL DESIGN

The effect of storage and processing of tricapryloylglycerol with increasing contents of pheophytin. Six 10-g lots of purified tricapryloylglycerol were each placed in 50-mL round-bottomed flasks. Pheophytin was added at a level of 30 ppm to two flasks and at a level of 200 ppm to two other flasks. The final two flasks contained control blank pheophytin preparations, added in an amount equivalent to that added to the 200 ppm samples. One control, 30 and 200 ppm sample were then placed at −40°C, while the remaining samples were placed in a closed cabinet at ambient temperature (23°C). After one week, all samples were processed, and a stability assessment was performed.

The effect of variation in exposure time and amount of bleaching clay. Six 15-g lots of purified canola oil were each placed in 50-mL round-bottomed flasks. Filtrol 105 clay was added to five of these, three at a level of 5% (w/w), and two at a level of 1% (w/w). Flasks containing oil and clay at a 1 and a 5% level were subjected to the bleaching conditions as described previously for a period of 60 min. Flasks containing oil and clay at a level of 1 and 5% and the flask containing oil alone were subjected to bleaching conditions for 30 min. The final flask, containing clay at a 1% level, was subjected to the bleaching conditions as described previously; however, heat was not applied. After the bleaching treatments, the oils were centrifuged and filtered through a 0.2-μm filter syringe (Baxter Canlab), and the stability was assessed as described previously.

An identical experiment was performed with purified tricapryloylglycerol instead of canola oil. Stability assessments were performed on 1:1 (w/w) mixtures of tricapryloylglycerol and linseed oil (used instead of canola oil to decrease length of induction period of mixed oil).

Effect of storage in the presence or absence of light of canola with added pheophytin prior to processing. Two experiments were conducted in this section, with the stability assessment for each experiment as described previously.

In the first experiment, purified canola oil with 60 ppm of added pheophytin was divided into two portions. One portion was stored at −40°C until processing as described above, while the other was stored in the dark at 52°C for 2 wk prior to processing and stability assessment.

In the second experiment, portions of purified canola oil with and without 60 ppm pheophytin were each divided into 4 × 2.5-g samples and placed in 30-mL beakers. Two of the beakers containing canola oil with added pheophytin and two of the beakers with canola alone were placed in a 60°C forced-air oven. The remaining beakers were placed in oil baths set at 60°C in a fume hood with fluorescent lamps at 934 lux. Aliquots of the oil from each treatment (0.04 g) were removed at appropriate time intervals, diluted with hexane, and the absorbance was determined at 234 nm.

RESULTS AND DISCUSSION

The effect of storage and processing of tricapryloylglycerol with increasing contents of pheophytin. Figure 1 presents the results of stability studies on canola oil to which tricapryloylglycerol was added that had been treated with different amounts of pheophytin and then stored at 23°C in the dark or frozen at −40°C for one week prior to pro-

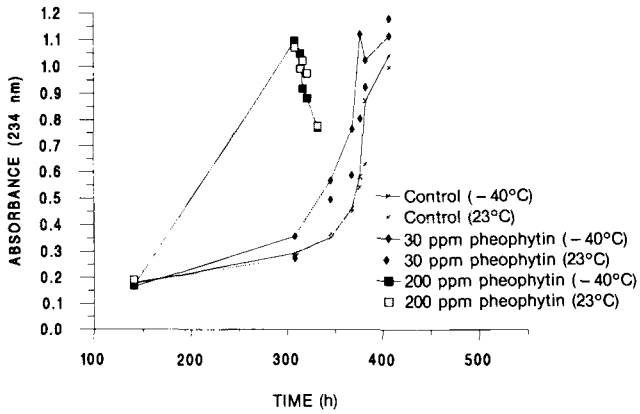

FIG. 1. Absorbance at 234 nm as a function of time of storage at 60°C for canola/tricapryloylglycerol blends.

cessing. The data show a trend toward decreased stability with increasing content of pheophytin before processing. The first data for the samples that contained 200 ppm pheophytin prior to processing were collected at 308 h when the breakdown of primary oxidation products exceeded production. The data indicated that these samples were significantly less stable than those containing 0 or 30 ppm pheophytin prior to processing, which were only at the end of their respective induction periods at this time (Table 1). These results are consistent with the production of prooxidant compounds from pheophytin during processing, which has been previously demonstrated in oils initially containing 60 ppm of pheophytin (15). The lack of effect upon storage at 23°C indicates that the production of these compounds occurred as a result of processing and was not due to a spontaneous breakdown of pheophytin during storage of the crude oil.

A statistical analysis of the data at 376 h for the oils that had 0 and 30 ppm pheophytin added prior to processing revealed that only the 30 ppm pheophytin sample that had been stored at −40°C differed significantly from the controls, suggesting that this level of addition may represent the lower limit for an observable effect.

Effect of variation in exposure time and amount of bleaching clay. Results of stability testing on canola oil, subjected to different bleaching regimes, showed that the use of increased amounts of clay from 1 to 5% (w/w) caused corresponding decreases in the stability of the oil (Fig. 2), which is supported by statistical analysis of the data collected at 164 h (Table 2). Additionally, at a level of 1% (w/w) clay, increased exposure time and temperature from 30 min at 23°C to 60 min at 104°C caused a significant decrease in stability. Examination of the stability of bleached tricapryloylglycerol/linseed oil mixtures revealed significantly lowered stabilities associated with exposure of the tricapryloylglycerol to any amount of bleaching clay. However, a temperature of 104°C was required for this effect (Fig. 3, Table 2).

Several reasons have been proposed for the decrease in the post-bleaching stability of oils. Boki *et al.* (16) found that bleaching with acid-activated clays can result in the increase of free fatty acids, which have been shown to be prooxidant in nature (17). However, no free fatty acids were detected in the bleached canola oil samples used in the present experiment. Autoxidation can be catalyzed by bleaching clay, and while hydroperoxides may be readsorbed, the presence of secondary oxidation products may decrease oil stability (7). As expected, no peroxides were found in the bleached canola oils. Determination of the anisidine value of these oils failed to show any similar trends between stability and the presence of any detectable aldehydes (as indicated by anisidine values, Table 3). In addition, such products would not be expected to be present when purified tricapryloylglycerol (a fully saturated triacylglycerol) was used.

Iron and phosphorus (indicative of phospholipid content) both affect the oxidative stability of an oil (18,19). However, no detectable levels of either of these compounds were found in the bleached canola oils (the levels of detection were 0.02 and 0.2 ppm, respectively).

Tocopherol levels in the bleached canola oils differed slightly, although only the two least stable oils (those

TABLE 1

Average Values of Absorbance at 234 nm After Storage at 60°C in the Dark for Samples Containing Tricapryloylglycerol That Contained Various Amounts of Pheophytin and Had Been Stored Under Different Storage Conditions Prior to Processing

Treatment		Storage at 60°C (h)	
Amount pheophytin added prior to processing (ppm)	Storage temperature of oil prior to processing (°C)	308	376
0	−40	0.293a[a] (0.019)[b]	0.583a (0.098)
0	23	0.291a (0.013)	0.543a (0.054)
30	−40	0.357a (0.121)	1.112b (0.026)
30	23	0.274a (0.025)	0.806a,b (0.218)
200	−40	1.096b (0.024)	
200	23	1.072b (0.064)	

[a]Values within a column sharing the same letter are not significantly different ($P < 0.05$).
[b]Values in parentheses are standard deviations of averages.

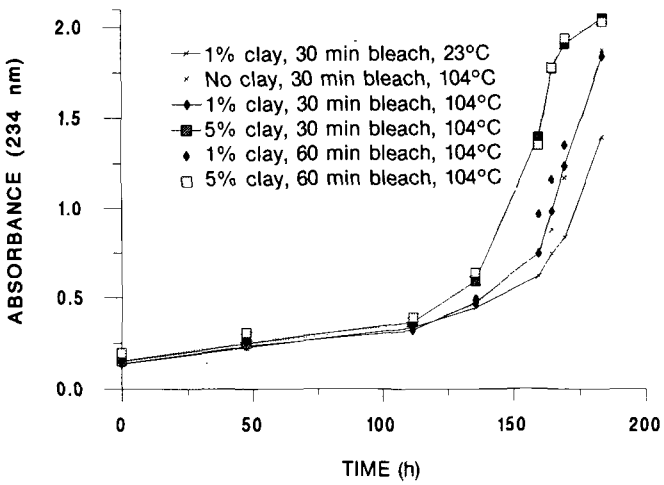

FIG. 2. Absorbance at 234 nm as a function of time of storage at 60°C of canola oil subjected to various bleaching treatments.

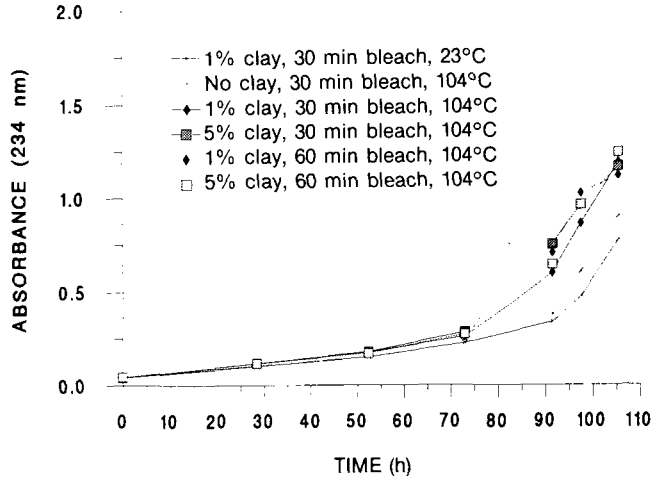

FIG. 3. Absorbance at 234 nm as a function of time of storage at 60°C for linseed/tricapryloylglycerol blends (the latter having been subjected to various bleaching treatments).

treated with 5% clay) showed lower amounts (Table 3). Tocopherol analysis of the tricapryloylglycerol could not detect any homologues of this compound (detection limit 5 ppm under the conditions utilized), and therefore, the varying stabilities of the tricapryloylglycerol/linseed oil blends could not be attributed to such an influence.

The most likely factor affecting the stability of the oils in this study was the content of free fatty acids because the detection limit of the assay used (0.3%, w/w) was higher than levels found to be influential in oil stability (0.02%, w/w) (17). Whatever the cause for the decrease in stability that was observed in various oils exposed to conditions involving greater amounts of bleaching clay [from 1 to 5% (w/w)], longer exposure times (from 30 to 60 min) and increased temperature (from 23 to 104°C), it is obvious that the more rigorous bleaching that would be required to reduce pigment content in oil from green seed would be expected to have a significant effect on the processed oil stability.

Effect of abusive storage of canola oil prior to processing. Purified canola oil, stored for 2 wk at 52°C in the dark, had less stability than the control oil stored at −40°C (Fig. 4). Oxidation in the oil stored at 52°C and the subsequent removal of primary autoxidation products through processing are indicated by the absorbance decrease from 0.466 to 0.223 at 234 nm of this sample before and after processing, respectively (Table 4). However, the anisidine values of the oil stored at 52°C increased during processing, revealing the production and survival of secondary oxidation products. The prooxidative effect of these compounds is shown by the increased anisidine value corresponding with increased oil instability. This

TABLE 2

Average Values of Absorbance at 234 nm After Storage at 60°C in the Dark for Samples Containing Canola Oil or Tricapryloylglycerol That Had Been Subjected to Various Bleaching Treatments

Treatment			Storage at 60°C (h)	
Amount of bleaching clay added to oil (% w/w)	Exposure time to clay (min)	Temperature of treatment (°C)	Canola (164)	Tricapryloylglycerol/linseed (97.5)
1	30	23	0.746a[a] (0.058)[b]	0.473a (0.000)
0	30	104	0.880a,b (0.001)	0.064a (0.125)
1	30	104	0.984a,b (0.075)	0.863b (0.067)
1	60	104	1.164b (0.031)	1.025b (0.054)
5	30	104	1.775c (0.256)	0.961b (0.001)
5	60	104	1.777c (0.057)	0.964b (0.075)

[a]Values within a column sharing the same letter are not significantly different ($P < 0.05$).
[b]Values in parentheses are standard deviations of averages.

TABLE 3

p-Anisidine Values and Tocopherol Contents of Canola Oil Exposed to Various Bleaching Treatments

Amount of bleaching clay added to oil (% w/w)	Exposure time to clay (min)	Temperature of treatment (°C)	*p*-Anisidine value	Tocopherol content (mg/100 g oil)
1	30	23	0.19	20.3
0	30	104	0.036	21.1
1	30	104	0.62	20.3
1	60	104	0.89	20.6
5	30	104	0.31	18.4
5	60	104	0.11	18.4

suggests that even in the absence of pheophytin, a decrease in the quality of canola oil from autoxidation during storage prior to processing can result in a processed oil with poor stability. Similar results have been reported with poor-quality oil from damaged soybeans (20).

Canola oil stored at 60°C was least stable when stored in the light in the presence of pheophytin, which is readily explained by the prophotooxidative effect of pheophytin (Fig. 5). Statistical analysis of the slopes of the lines from 0 to 77 h showed this treatment to be significantly different from the remaining three, among which no significant differences existed. Although the slopes of the curves representing oils stored in the dark were not statistically different, the trend toward increased stability of the oil with added pheophytin confirms the antiautoxidative effect of pheophytin in oils stored in the dark that has been noted by other researchers (21), and which was explained by a competition phenomenon (22). It is apparent that if crude canola oil is exposed to conditions, such as light, that will enhance the prooxidative effect of chlorophyll derivatives, oxidation will occur, resulting in the production of secondary autoxidation products that, if not removed by processing, will result in an oil of lower stability.

The results of this study show that the causes of instability of processed oil from green seed are probably many and complex. The action of the bleaching clay is important, particularly with higher levels of clay and longer times of treatment, which were shown to have a significant detrimental effect on oil stability. The addition of pheophytin to oil prior to processing decreased the processed oil's stability, suggesting the production of prooxidative compounds as a result of exposure of pheophytin to processing conditions. Finally, the quality of an oil prior to processing is important, as shown by the decreased stability of processed oxidized oil. Research is needed into the identity of prooxidation compounds produced through the processing of pheophytin, and the causes behind the alteration of stability as caused by intensified

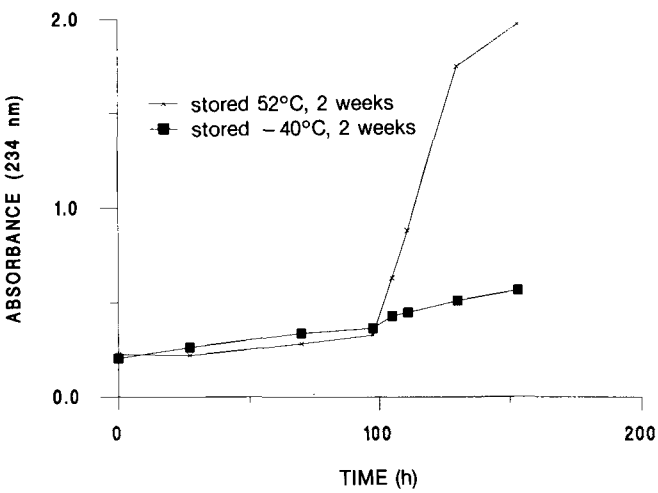

FIG. 4. Absorbance at 234 nm as a function of time of storage at 60°C for canola oil stored under various conditions of temperature prior to processing.

TABLE 4

Anisidine Values and Absorbance at 234 nm of Canola Oils Processed After Storage Under Two Different Conditions

	Absorbance (234 nm)	Anisidine value
Stored 2 wk (−40°C)		
Before processing		2.76
After processing	0.201	0.40
Stored 2 wk (52°C)		
Before processing	0.466	12.4
After processing	0.223	61.0

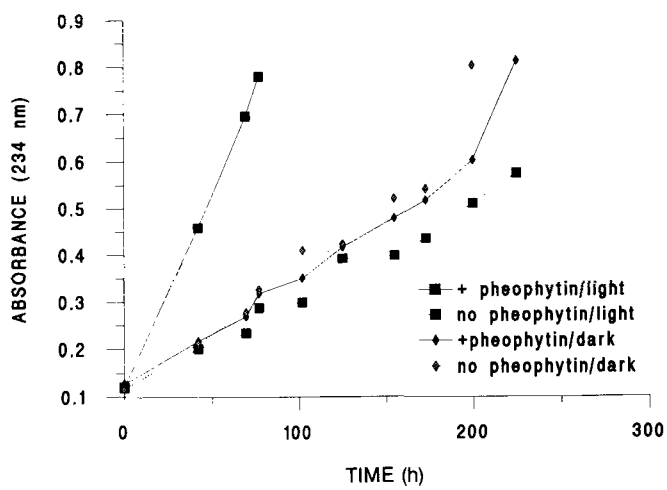

FIG. 5. Absorbance at 234 nm as a function of time of storage at 60°C for canola oil stored ± pheophytin in the light or dark.

bleaching conditions. The importance of protecting oils high in chlorophyll derivatives from light, prior to their removal by bleaching, has previously been noted by other researchers and has been substantiated by the results of this study.

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