Chlorophylls and Vegetable Oils

Tan Yew Ai*

RINGKASAN

Kertas ini menyingkap semula pengetahuan masakini mengenai klorofil dan hasil degradasi klorofil seperti feofitin dalam minyak sayuran termasuk minyak sawit. Dibincangkan juga mengenai adanya sebatian seperti ini dalam minyak, fungsinya dalam kestabilan oksidatif minyak, paras proses oksidasi berlaku dan kaedah-kaedah yang ada untuk mengukurnya. Akhir sekali, peranan klorofil dan bahan-bahan berkaitan minyak sawit dibincangkan termasuk kajian-kajian yang boleh dilakukan untuk mempastikan peranannya dalam kestabilan oksidatif minyak sawit mentah dan penapisan.

INTRODUCTION

One of the major differences between animals and green plants is the ability of the latter to transform electromagnetic energy (visible light) into chemical energy to drive synthetic reactions. This ability is known as photosynthesis and chlorophylls are the major photosynthetic pigments which enable green plants to affect this energy transformation. Unlike certain food components such as carotenes and tocopherols, chlorophylls do not seem to perform any function of metabolic importance in animals consuming them.

Structurally, the chlorophyll (CHL) molecule contains a porphyrin (tetrapyrrole) nucleus with a chelated magnesium atom in the centre (*Figure 1*). An isoprenoid alcohol (phytol) is attached to the porphyrin ring at position 7. There are at least five types of chlorophylls *i.e.* chlorophyll a,b,c,d and e, in plants. Each type has the same basic structure but differs in the nature of the aliphatic side chain attached to the porphyrin nucleus at position 3. Chlorophyll a and b (CHL a, CHL b) occur in higher plants and CHL c, d and e are only found in algae. Yet other types of CHL are present in certain bacteria.

The loss of the magnesium atom from the centre of the porphyrin ring results in the respective pheophytins (PHY). Further loss of phytol by hydrolysis of the ester leads to the formation of pheophorbides (PHO) (*Figure 1*). On the other hand, should the CHL molecules lose the phytol moiety enzymically, the corresponding degradation products are known as chlorophyllides. Phyropheophytins arise from the loss of the pheophytin (PHY) C10 carbomethyoxy group (*Figure 2*). Other more complex breakdown products of CHL are discussed by Jackson (1976).

Chlorophylls and their derivatives are also photosensitizers. These chemical species are capable of absorbing light, to activate or "sensitize" either the unsatu-

* *Chemistry and Technology Division*
Palm Oil Research Institute of Malaysia

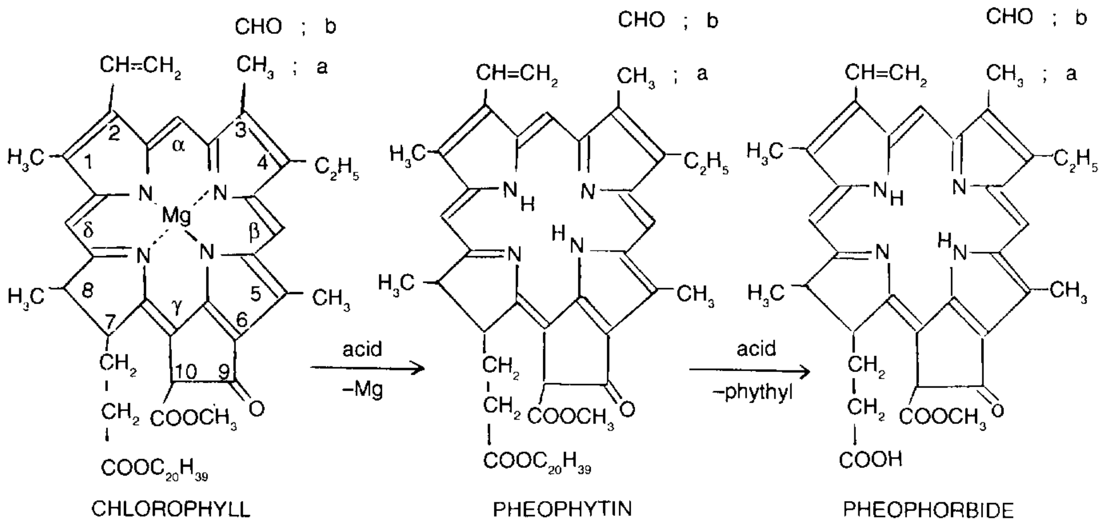


Figure 1. Structure of chlorophyll, pheophytin and pheophorbide.

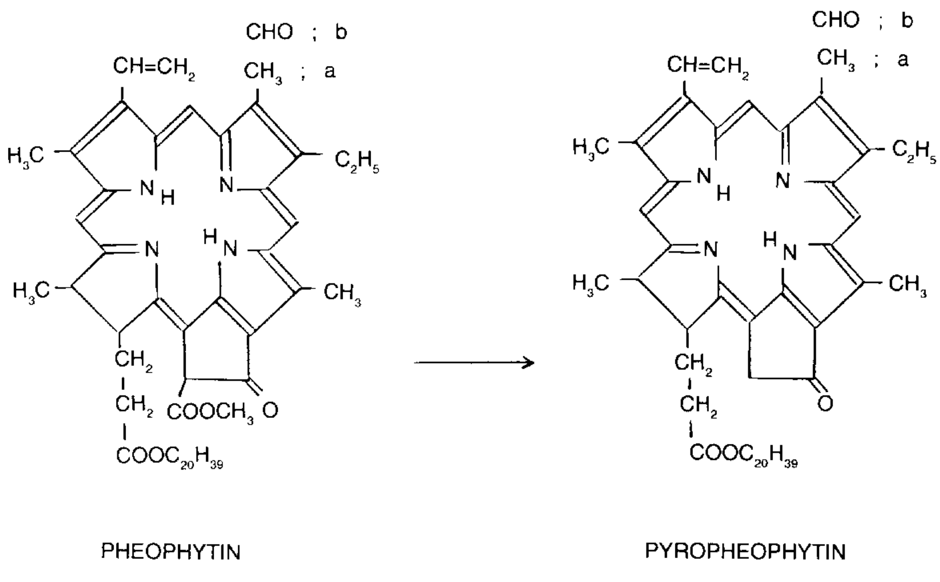


Figure 2. Structure of pyropheophytin.

rated oils or molecular oxygen to induce photosensitized oxidation. They themselves do not undergo oxidation on absorption of light.

CHLOROPHYLLS AND DERIVATIVES IN VEGETABLE OILS

Chlorophylls and chlorophyll derivatives are a class of pigments occurring in small quantities in vegetable oil products. The major pigments present in oils include the carotenoids and xanthophylls. The presence of CHL in oils is considered undesirable because of their adverse effects on oxidative deterioration, hydrogenation and bleachability (Coe, 1938; Abraham and Deman 1963; Koritala, 1973 and Dahlen, 1973). Usually more efforts with resultant higher costs have to be put in to bleach, heat treat or hydrogenate vegetable oils containing CHL before acceptable colour levels are attained.

Most vegetable oils, contain CHL. In rapeseeds, the CHL content is an important criterion in the measurement of maturity/ripeness. It is in fact used as a quality index for rapeseeds (Dahlen, 1973). Since the extraction of rapeseed oil, or for that matter oil from any other fruits or seeds containing CHL, will invariably result in some CHL being extracted together with the oil, the role of CHL in determining quality of the final oil is clearly important.

Canola, the trademark of the Canola Council of Canada, refers to rapeseeds and products from varieties of *Brassica napus* and *Brassica campestris*. These two species of rape are low in erucic acid and glucosinolates. It is well known that maturity or greenness is the major degrading factor in Canadian rapeseed. It is also related to the major problem in the Canadian crushing industry, as oil extracted from green seeds contain such quantities of CHL as to cause bleachability problems. Niewiadomski *et al.* (1965) found no CHL a in rapeseed oil but reported that PHY a ranged from 17.99 – 25.65 mg/kg. They found 0.14 – 1.79 mg/kg CHL b in some of the rapeseed oils and PHY b was estimated to be around 0.52 – 6.15 mg/kg. A study by Davies *et al.* (1990) showed that crude rapeseed oil (RSO) can contain CHL pigments in the region of 5 – 70 mg/kg (or ppm).

Soya bean oil (SBO) too can contain a significant quantity of CHL. Pritchett *et al.* (1947) found 1500 μg/kg (or ppb) of CHL in normal crude SBO and 15 μg/kg in the refined oil.

An investigation by Ikemefuna and Adamson (1984) on the CHL and carotenoid changes in palm fruit *Elaeis guineensis,* indicated that CHL did not disappear completely in the ripe palm fruits. They showed that green, mature and ripe fruits of the Tenera and Dura varieties contained CHL a and CHL b in varying amounts (*Table 1*). Strecker *et al.* (1990) in their paper on physically refined oils, gave a value of 827 ppb for CPO and 30 ppb for crude palm kernel oil. Their method of analysis was not mentioned but they found no CHL in the refined products of palm and palm kernel oil. *Table 2* is a summary of their results.

A subsequent paper by Usuki *et al.* (1984a) which reported on the residual amounts of CHL and PHY in refined edible oils showed that refined palm olein contained as much as 582.9 μg/kg of total CHL consisting of CHL a, CHL b, PHY a and PHY b. They quantified total CHL in nine different types of refined edible oils by a fluorometric method and found that the total CHL content increased in the order of soya bean < corn < rapeseed < cottonseed < safflower < palm olein (*Ta-*

ble 3). The mean ratios of the a type to b type chlorophyll were similar in different oils with the exception of sesame oil. The mean ratio of a:b was 70:30%. An important point to note is that the content of PHY a is the highest at about 67% of total CHL. This higher level of PHY in oils is further confirmed by Rahmani and Csallany (1985) and Fraser and Frankl (1985). They reported that in SBO and olive oil, PHY a was present as

TABLE 1 . CHLOROPHYLLS (mg/100g) OF TWO FRUIT FORMS OF *E. guineensis* AT THREE STAGES OF DEVELOPMENT (Ikemefuna and Adamson, 1984)

Pigment		Tenera			Dura		
		*Green	*Mature	*Ripe	*Green	*Mature	*Ripe
Chlorophyll a	Mean	2.89	2.07	0.43	2.65	2.27	0.24
	Range	1.2-4.8	0.3-3.4	0.03-0.73	1.3-5.4	1.6-3.4	0.07-0.37
Chlorophyll b	Mean	1.86	1.53	0.73	1.9	1.18	0.46
	Range	0.8-3.3	0.3-2.0	0.03-1.3	1.4-3.5	0.5-1.7	0.13-0.72

*Green : 1 - 2 months
Mature : 3 - 4 months
Ripe : 5 - 6 months

TABLE 2. CHLOROPHYLL (ppb) CONTENT OF NINE VEGETABLE OILS (Strecker *et al.*, 1990)

Oil Type	Crude	Alkali Refined	Physically Refined
Sunflowerseed	692	40	41
Canola	16,880	2.5	1.2
Soya bean	576	4	8
Peanut	90	8	10
Rice Bran	9,850	40	50
Safflower	213	8	8
Coconut	148	5	5
Palm kernel	30	-	-
Palm oil	827	-	-

TABLE 3. CONTENTS OF CHLOROPHYLLS (μg/kg) AND PHEOPHYTINS IN SEVERAL REFINED EDIBLE OILS (Usuki *et al.*, 1984a)

Oil	Total	Chl a	Chl b	Phya	Phy b
Soya bean	116.1	2.0	13.3	73.1	27.7
Corn	173.8	0	18.9	114.4	40.5
Rapeseed	219.6	15.2	30.6	131.5	42.3
Cottonseed	278.8	9.7	39.8	184.9	44.3
Safflower	311.9	31.7	60.2	167.2	52.8
Palm Olein	582.9	30.3	113.8	341.2	97.6

the predominant break-down product of CHL. The CHL are presumed to be lost during the early stage of oil extraction. This presumption was confirmed by Usuki and Endo (1987) when they investigated the compositional changes in CHL and PHY during extraction and refining of RSO. They concluded that levels of PHY in refined edible oils can be kept low if the extraction is carried out at a lower temperature with minimal exposure to heat. CHL, present at higher levels in the seeds, are bound to protein whereas PHY exist in the free form. Therefore unlike CHL, PHY is predominantly extracted into the oil, especially so during hexane extraction. Since CHL are converted to PHY by losing magnesium under acidic conditions, the citric or phosphoric acid used in the degumming process will also cause conversion of part of the CHL to PHY.

EFFECTS OF CHL AND PHY IN VEGETABLE OILS

Once CHL get into the oil, they are difficult to remove by conventional alkali treatment and bleaching processes (Dahlen, 1973). If the quantity extracted into the oil is significant, its subsequent removal will increase the cost of refining. As far back as 1938, it was suggested (Coe, 1938) that rancidity in vegetable oils might be correlated with the presence of CHL which acting as photosensitizers liberate free hydrogen under the photochemical reaction. In addition, Gaffron (1926), reported that the quantity of peroxides formed in oil was many times the amount equivalent of CHL. This meant that only a trace amount of CHL was needed to bring about molecular oxidation or rancidity in vegetable oils. Since the time of Coe, many reports on the correlation of CHL level with oil deterioration have been published (Lee, 1955, Endo *et al.*, 1984a, 1984c, 1985a; Fakourelis *et al.*, 1987). In brief, all these investigations implicated CHL and their derivatives as prooxidants because of their photosensitizing activities in the oil.

It is known that CHL act as photosensitizers to generate singlet oxygen ($^1O_2$) from triplet oxygen ($^3O_2$). Singlet oxygen then initiates the oxidation process by the production of free radicals. Evidence showing the importance of photosensitized oxidation in initiating or propagating normal free radical autoxidation of unsaturated fats was reported by Rawls and Van Santen (1970), Clements *et al.* (1973), Chan (1977) and Terao and Matsushita (1977). Rawls and Van Santen and Clements *et al.* reported that exposure to diffused daylight or artificial light caused a marked acceleration in the deterioration of unsaturated oils. They postulated that deterioration was probably sensitized by chromophoric impurities in oils, especially residual natural pigments such as CHL, PHY, myoglobin and porphyrins. These chromophores absorb strongly in the visible or near UV light. They suggested that in contrast to thermal oxidation, light initiated deterioration did not involve free radicals but rather singlet oxygen ($^1O_2$) generated by the transfer of excitation energy from excited chromophoric impurities to oxygen as shown by *Equation 1.*

Single oxygen reacts rapidly with the carbon adjacent to the double bond of fatty acids to give hydroperoxides via the molecular process shown by *Equation 2* (Foote, 1968; Kaplan, 1971).

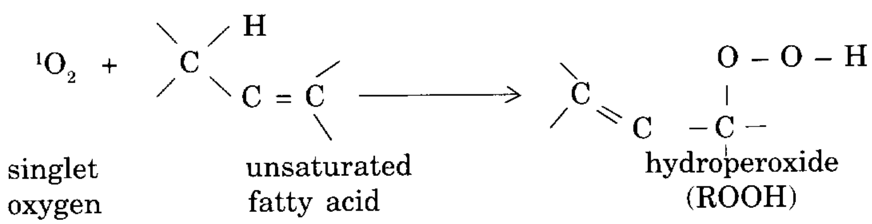
The hydroperoxides can thermally cleave even at room temperature, thereby initiating the conventional free radical autoxidation to produce more hydroperoxides (*Equation 3*) (Chan 1987).

The role of singlet oxygen in initiating autoxidation was outlined by Rawls and Van Santen (1970). They established that it was the primary source of original hydroperoxides produced during autooxidation or photooxidation. Since as low a concentration as 200 μg/kg (or ppb) of oxidation products can result in significant off-flavours and odours, (Labuza, 1972), a knowledge of the initial cause of hydroperoxide formation is all important. It has been established that photooxidation is one of the three pathways for the production of hydroperoxides from unsaturated fatty acids. The other two pathways are autoxidation and enzyme-catalyzed oxidation.

While investigating the photosensitizing activities of CHL in oils Rawls and Van Santen (1970) and Clements *et al.* (1973) found that singlet oxygen oxidation could be prevented by ß-carotene and triethylamine. These two compounds are known to quench or deactivate excited singlet oxygen to the ground state, *i.e.* triplet oxygen (*Equation 4*).

Carlsson *et al.* (1976), in a study on effects of singlet oxygen quenchers, concluded that only the most efficient singlet oxygen quenchers can provide protection. The quenchers have to be present at about 0.01% by weight of the oil because of the high rate of singlet oxygen attack on

$$\underset{\text{chromophore}}{\mathrm{Ch}} \xrightarrow[\text{(light)}]{hv} \underset{\substack{\text{excited}\\ \text{chromophore}}}{\{\mathrm{Ch}\}^*} \xrightarrow[\substack{\text{triplet}\\ \text{oxygen}}]{^3O_2} \mathrm{Ch} + \underset{\substack{\text{singlet}\\ \text{oxygen}}}{^1O_2} \qquad \text{Equation 1}$$



Equation 2

allylic methylene groups. ß-Carotene and α-tocopherol (Vitamin E) are additives which meet this quenching criterion. However, the same workers also showed that α-tocopherol itself underwent a relatively rapid oxidation, to give hydroperoxide products when exposed to singlet oxygen in oil solutions. Therefore its singlet oxygen quenching effect is somewhat offset by its own oxidation. On the whole, the group concluded that singlet oxygen-initiated deterioration is appreciably more difficult to retard than the peroxy radical processes.

The role of carotenoids as a singlet oxygen quencher is not as clear-cut as that of α-tocopherol. It is known that ß-carotene can quench most polynuclear aromatics (Herkstroeter, 1975). There is also evidence that they do protect lipid against photosentisized oxidation by quenching singlet oxygen (Carlsson *et al.*, 1970; Lee and Min, 1988). Foote *et al.* (1970) estimated that for every molecule of ß-carotene consumed, at least one thousand molecules of singlet oxygen were quenched. The energy transfer mechanism for this quenching effect is shown by *Equation 5.*

Additional evidence by Fujimori and Livingston (1957) and Krinsky (1979) showed that carotenoids can also react with the triplet state excited sensitizers by a similar energy transfer mechanism shown by *Equation 6.*

However, work by Lee and Min (1988) showed that carotene cannot react with CHL by the mechanism shown by *Equation 6,* although carotene can quench singlet oxygen produced by the photosensitizing activities of CHL.

Further work on carotenes suggested that they retard photooxidation by quenching singlet oxygen but at the same time are also susceptible to free radical autoxidation. In the study on the prooxidant effect of carotenoids on the oxidation of refined edible oils, Suzuki *et al.* (1989) found that ß-carotene has the ability to accelerate the deterioration of oils kept in the dark at 30°C. The deterioration was especially significant if the oils were highly oxidized before the addition of ß-carotene. They concluded that the intermediate oxidation products of ß-carotene, the formation of which were promoted or caused by the oxidation products of oils, acted as initiators for lipid oxidation. Other studies on the prooxidant effects of carotene on fats and oils were reported by Emanuel and Lyaskovskaya (1976). The review by Frankel (1989) also cautioned that the role of ß-carotene

$$\underset{\text{hydroperoxide}}{2\ \mathrm{ROOH}} \xrightarrow[\text{cleavage}]{\text{thermal}} \mathrm{RO^{\bullet}} + \mathrm{H_2O} + \mathrm{RO_2^{\bullet}} \qquad \text{Equation 3}$$

$$\mathrm{RO_2^{\bullet}} \xrightarrow{+\ \mathrm{RH}\ \text{(unsaturated lipid molecule)}} \mathrm{R\ OOH} + \mathrm{R^{\bullet}}$$

$$\underset{\text{singlet oxygen}}{{}^1O_2} + \text{Quencher} \longrightarrow \underset{\text{triplet oxygen}}{{}^3O_2} + \text{Quencher} \qquad \text{Equation 4}$$

as a singlet oxygen quencher is complicated by its susceptibility to free radical autoxidation. By its preferential oxidation the oils are thus protected until ß-carotene itself is completely oxidized.

Other researchers who observed the prooxidant activities of carotenoids and/or their oxidation products included Olcott (1934), Lee (1955), Arya *et al.* (1979) and Faria and Mukai (1983). In addition Kiritsakis and Dugan (1985) found that carotenes and tocopherols were apparently destroyed more rapidly when chlorophyll was present. Therefore the role of carotenoids in stabilizing edible oils is still controversial. The evidence at this stage suggests that tocopherols effectively protect ß-carotene against free radicals autoxidation and hence, for ß-carotene to act effectively as a quencher, the presence of tocopherols is required.

There are, of course, other related molecules which have been reported as photosensitizers in oils. Pheophytin, chlorophyllin and protoporphyrin are but a few examples (Carlsson *et al.*, 1976; Terao and Matsushita (1977). Pheophytins (PHY) and pheophorbides (PHO) have in fact been reported to be more potent prooxidants than CHL (Endo *et al.*, 1984a, 1984b, Usuki *et al.*, 1984b). Since a considerable amount of PHY is found in commercial vegetable oils, the level of PHY must be taken into account when considering the oxidative stability of edible oils. However, Usuki *et al.* (1984a) had presumed that no PHO was present in the final refined product.

Another interesting effect of CHL in oils was reported by Endo *et al.* (1985a, 1985b). They claimed that CHL in vegetable oils stored in the dark at low temperature could act as an antioxidant to prevent their oxidative deterioration. This antioxidant effect of CHL was also reported by Gutierrez-Rosales *et al.* (1992). Endo *et al.* postulated that CHL may act as a hydrogen donor to break the chain reaction in autoxidation as shown by *Equations 7* and *8*.

In other words, in the dark CHL and PHL may act as radical scavengers by trapping the peroxy and other free radicals to inhibit autoxidation of oils. However Endo *et al.* (1985a, 1985b) did concede that the presence of tocopherols can affect the antioxidative activities of CHL and PHY. In addition, they found that the extent of antioxidant activities of these pigments/substances depended on the content of polyunsaturated fatty acids of oils. This was supported by their observation that better activities were noted in SBO with 61% linoleate and linolenate as compared to RSO with 34%.

$$\underset{\text{singlet oxygen}}{{}^1O_2} + \text{Carotene} \longrightarrow \underset{\text{triplet oxygen}}{{}^3O_2} + {}^3\text{Carotene} \qquad \text{Equation 5}$$

$$\underset{\text{(Excited state)}}{{}^3\text{Sensitizer}} + \text{Carotene} \longrightarrow \underset{\text{(ground state)}}{\text{Sensitizer}} + {}^3\text{Carotene} \qquad \text{Equation 6}$$

Besides being implicated in the oxidative deterioration of oils, CHL are also known to affect the hydrogenation rate of refined and bleached canola oil. Abraham and Deman (1963) reported that CHL slowed down the rate of both selective and non-selective hydrogenation and that addition of 1 mg/kg of CHL was sufficient to decrease solid fat content under non-selective conditions. CHL also reduced trans isomer content under non-selective conditions. At levels of CHL addition between 1-10 mg/kg, they found that the non-selective conditions resulted in a greater decrease of linolenic acid (18:3) and faster production of stearic acid (18:0) than selective conditions.

In the investigation on the selective hydrogenation of soya bean oil, Koritala (1974) found that oils containing PHY turned green after hydrogenation. This was presumably attributed to the formation of copper chlorophyll. Some of this green colour was not completely removed by post-bleaching (Mag, 1983). It was also observed that PHY increased the time for hydrogenation of the oil.

LEVELS OF CHL AND CHL DERIVATIVES WHICH AFFECT OXIDATIVE STABILITY IN VEGETABLE OILS

There is now voluminous evidence as to the effects of CHL and their derivatives on the oxidative stability of vegetable oils. However the important question is: What levels of CHL and their derivatives will be required to have an effect on the oxidative stability of both crude and refined vegetable oils?

We are especially concerned with the level of CHL and CHL derivatives in crude palm oil and palm oil products. Oxidation in oils is regulated by a myriad of components such as carotenoids, tocopherols, CHL, CHL derivatives and other naturally occurring constituents. Palm oil has almost all of these components. In fact CPO is distinguished from the other common vegetable oils by its extremely high carotene content (500-800 mg/kg) and comparatively low tocopherol content (500-900 mg/kg). The presence of CHL in CPO has been overlooked because of the high carotene content which masked the green colour of CHL. Clements *et al.* (1973) recommended besides the removal of CHL, the use of efficient yellow filter materials to prevent light-induced deterioration of oils. Thus on the surface there really does not seem to be a problem with CPO since it already possesses built-in filters in the form of its high carotene content. However, doubts on the protective effect of carotene on the oxidative stability of CPO now arise because of the cumulative evidence on the prooxidative effects of carotenes coupled with the antioxidative effects of CHL in oils stored in the dark

$$ROO^{\bullet} + CHL \longrightarrow ROO{:}^{(-)}\ CHL.^{(+)} \qquad \text{Equation 7}$$

$$ROO{:}^{(-)}\ CHL.^{(+)} + ROO^{\bullet} \longrightarrow \text{Inactive products} \qquad \text{Equation 8}$$

where $ROO^{\bullet}$ = peroxy radicals

(Suzuki *et al.*, 1989, Frankel, 1989 and Endo *et al.*, 1985a, 1985b). This evidence is especially significant to the palm oil industry since CPO is stored in closed storage tanks which exclude light.

As previously mentioned, Gaffron (1926) inferred that only a trace amount of CHL was necessary to affect oxidative stability of oils. Much later, in 1979, Frankel *et al.* reported that only traces of photosensitizers in SBO and other vegetable oil esters could catalyze the oxidation of linoleate by singlet oxygen.

In the earlier study of Coe (1938), it was observed that addition of 0-10 mg of CHL to 25cc of oil brought about a rapid increase in rancidity. M. Higuchi (1983), in his paper on quality control in oil refineries, indicated in one of his tables that at 20°C and 700 lux, as little as 100μg/kg of CHL in SBO stored for two weeks can affect the oil's stability and flavour. In the same paper, he cited that 200μg/kg of CHL in RSO could affect its oxidative stability. Such observations emphasize the need for efficient CHL removal from vegetable oils.

Subsequently, the Japanese researchers Endo and his co-workers published several papers on photoxidation and CHL (Endo *et al.*, 1984a, 1984b and 1984c). In their studies they exposed methyl linoleate (ML) containing about 200 mg/kg of CHL or PHY to light of 500μW/cm^2 sec at 0°C and found that photodegradation products of CHL could promote the oxidation of oils. They then added CHL a, CHL b, PHY a and PHY b in the range of 0.2-200 mg/kg to ML and irradiated the mixtures at 0°C and 40°C at five different light intensities of 0, 10, 50, 100 and 500 μW/cm^2 sec. Their experiments showed that the addition of CHL a or CHL b at 0°C at amounts exceeding 2 mg/kg catalyzed oxidation of ML. No oxidation was observed at 0.2 mg/kg. However, Endo *et al.* (1985a, 1985b) later reported that a concentrations of 2 mg/kg of both CHL and PHY retarded the autoxidation of ML only slightly when the ML was placed in the dark at 30°C. Usuki, a co-worker of Endo, studied the effects of CHL and PHY on photooxidation of triglycerides, and the alteration of CHL during photooxidation. In his paper (Usuki *et al.*, 1984b), it was reported that a pigment mixture (CHL a: CHL b: PHY a: PHY b=1:3:10:3) of 821μg/kg in the oil remained at almost the same initial concentration after 310 hours of autoxidation under room fluorescent light and at room temperature. This implies that the pigments especially PHY a and b, were relatively stable during oil oxidation. At a level of 750 μg/kg of each pigment added to photosensitizer-free RSO, an increase in peroxide value (PV) from 2 to 35 meq/kg was observed after 90 hours of exposure to light at room temperature.

In another study, Usuki *et al.* (1984a) investigated the effect of total CHL (CHLa + CHLb + PHYa + PHYb) at concentrations of 39, 233, 425, 623 and 821μg/kg on refined SBO stored under room light at 15°C. They found that the PV increased from 2 to more than 10 meq/kg in all samples tested. They also noted a similar magnitude of PV increase in refined RSO with total CHL of 171 μg/kg.

The study by Kiritsakis and Dugan (1985) showed that at a CHL concentration of 4-6 mg/kg of oil and exposure to light of 4500 lux and temperature of 30 ± 2°C, the rate of photooxidation of olive oil was increased. Fakourelis *et al.* (1987) added 0, 2 and 4 mg of CHL to 1 kg each of purified olive oil, stored the mixtures under light of 4000 lux and 25°C and analyzed the peroxide contents at the end of the experiment. Their results indicated that the presence of CHL in the oil resulted in higher PV formation and greater oxygen disappearance in the head space above the oil.

METHODS FOR CHL AND CHL DERIVATIVES DETERMINATION

Chlorophylls occur in the chloroplast in relatively large amounts and are often bound loosely to protein. They are readily extracted in lipid solvents such as acetone and ether. Both CHL and carotenoids have rather similar solubilities in the extracting and chromatographic solvents used. Ironically, this similarity has also made it difficult to quantify CHL in the presence of carotenoids as in the special case of CPO. Compared to carotenoids, CHL is the more photo/light labile component and great care has to be taken to ensure minimal exposure to light when analyzing CHL.

Extraction followed by spectrophotometric analysis is the usual procedure employed in the determination of CHL in oil products. In the spectrophotometric methods, only the apparent CHL or compounds having an absorption maximum in the range of 660-670 nm are determined. Loss of magnesium from CHL as in the case of PHY, results in a two times reduction in intensity of the absorption band near 670 nm for CHL a and b. The understanding among analysts is that the PHY rather than the CHL are probably measured in spectrophotometric methods. The method adopted by the American Oil Chemist' Society (AOCS) Official Method Cc 13d-55 1989) is one such method. It permits determination of CHL in vegetable oils at ppm levels from spectrophotometric measurements at 630, 670 nm and 710 nm. The method is not applicable to hydrogenated oils, deodorized oils and finished oil products since in these processed oils, CHL absorption band at 670 nm is absent. The ISO Method (1992) is also quite similar to the AOCS method but wavelengths at 625.5, 665.5 and 705.5 are taken instead. This method is calibrated against chlorophyll a and, again measures only the 'apparent chlorophyll' which includes related pigments with absorption near 665nm, chlorophyll a and b and their respective degradation products.

The method adopted by the International Union of Pure and Applied Chemistry (IUPAC) is also based on spectrophotometry. It is only suitable for the determination of quantities of total CHL, expressed as PHY a, which occur in oils at greater than 1 mg/kg (or 1 ppm).

As mentioned earlier, the AOCS Cc 13d-55, only measures the apparent CHL content of oils. While adequate for general purposes, it is nevertheless inaccurate and does not meet the requirements of analytical chemists. Daun's (1982) study on canola oil emphasized the deficiency of the AOCS method and he proposed modifications to include calibration for major PHY-based pigments which were present in the oil.

CHL is one of the many organic compounds which exhibit fluorescence. This fluorescence property of CHL forms the basis of HPLC (High-Performance Liquid Chromatography) analysis using fluorescence detectors. Determination of CHL and their derivatives in oil by HPLC is described by Eskins and Dutton (1988), Daun and Thorsteinson (1989), Rahmani and Csallany (1985), Fraser and Fiankl (1985), Jordan and Mudderman (1985), Aitzemuller (1989), Pfannkoch and Gill (1990) and Davies *et al.* (1990).

Mixtures of CHL a and CHL b in acetone can be determined from the independent fluorescence activities of the two. In 1972, White *et al.* evaluated a study on the accuracy of the spectrophotofluorometric or, fluorometric method, for the estimation of CHL in phytoplanktons. CHL remaining in refined edible oils and fats are usually below the limit of detection by conventional spectrophotometric techniques. Noting that the detection limit by fluorometry is about 1000 times lower than absorption spectroscopy, Usuki *et al.* (1983) modified the fluorometric method by White for the estimation of both CHL and PHY in refined edible fats and oils. The method is simple and involves the dissolution of 3.64 g of sample oil in 16 ml of acetone and measurement of fluorescence intensities at four different sets of excitation and emission wavelengths.

DISCUSSION

Published papers have clearly shown that trace amounts of photosensitizers (CHL and their derivatives) can accelerate oxidative deterioration of oils. Various workers have shown that the μg/kg (or ppb) level is enough to cause significant oxidation even in oils with high tocopherol content such as SBO and RSO.

Carotenoids and tocopherols are known singlet oxygen quenchers but both are easily destroyed in the presence of CHL. While protecting the oil, they are at the same time susceptible to autoxidation. Carotenes in fact have to be protected by tocopherols against free radical autoxidation. On the other hand, CHL especially PHY are stable to oxidation.

Ikemefuna and Adamson (1984) and Usuki *et al.* (1984a) have shown that CHL are present at ppb levels in both crude and refined palm oil products. These levels are insignificant indeed compared to those of carotenoids and tocopherols. Many other factors have to be taken into account when considering the subject of autoxidation of edible oils in both light and dark conditions. Oxygen, temperature, fatty acids and triglycerides composition, tocopherols, carotenoids, chlorophylls, conjugated oxodienes, metals and various other minor components affect oxidative stability. It is the delicate balance of these factors which will determine oxidative behaviour of the final product.

In the production of refined palm oil, the industry uses adsorbents to remove carotenoids on the understanding that the CHL are probably removed at the same time. The question is how efficient are these adsorbents in the removal of CHL and their derivatives? The level of 581 µg of total CHL per kg of refined palm olein reported by Usuki *et al.* (1984a) was higher than those reported for refined SBO and RSO. It follows from reason that CPO would contain more CHL than crude SBO and crude RSO, if the technologies used in the three oils removed similar proportions of CHL. On the other hand, if, to begin with, CPO has lower CHL content than the other two crude oils, then it follows that the higher CHL level in refined palm oil would be due to a less efficient refining technology in removing CHL than those used in the processing of the other two oils. Whatever the case may be, it can be readily determined by experimental studies. Accordingly, the following programme of research was initiated:-

(1) To develop a new method based on LASER technology for the analysis of CHL in palm oil.
(2) Study of CHL content in both crude and refined palm oil and palm oil products.
(3) To study if there is any correlation between CHL level and the oxidative stability of crude and refined palm oils.

(4) To use, if applicable, CHL level as an indicator of quality in CPO, and
(5) To develop, if possible, a predictive test for stability of refined palm oil based on CHL data.

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