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VALUABLE MINOR CONSTITUENTS OF COMMERCIAL RED PALM OLEIN: CAROTENOIDS, VITAMIN E, UBIQUINONES AND STEROLS

Keywords: Commercial red palm olein, carotenoids, sterols, vitamin E, coenzyme Q_{10}, vitamin A equivalent, vitamin E equivalent, recommended daily allowances.

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The important minor constituents of crude palm oil (CPO) – carotenoids, vitamin E, ubiquinones and sterols are also preserved in the commercial red palm olein (CRPo). The average carotene content was found to be about 665 ppm. The high-performance liquid chromatography (HPLC) profile of carotenoids showed that all the 11 types of carotenoids of CPO were preserved in CRPo, α- and β-carotene still constitute about 80% of the total carotenoids. Xanthophylls: dehydro-retinal, ξ-caroten-dione and β-caroten-5,6-epoxide were also tentatively identified based on their UV spectra and elution order. The vitamin E content was in the range of 717-863 ppm, consisting of α-tocopherol (19%), α-tocotrienol (29%), γ-tocotrienol (41%) and δ-tocotrienol (10%). The sterol content is in the range of 325-365 ppm consisting of β-sitosterol (59%), campesterol (22%), stigmasterol (17%) and cholesterol (< 2.6%). Coenzyme Q_{10} was detected in CRPo with a concentration range of 18-25 ppm. The fatty acid composition indicates that CRPo has 46.7% monounsaturated, 12.8% polyunsaturated and 40.5% saturated fats.

INTRODUCTION

CRPo is an unconventional oil produced from CPO through a new process in which the deacidification and deodorization are carried out using molecular distillation under milder conditions (Ooi,1996). This preserves more than 80% of the carotenoids and vitamin E (Choo *et al.*, 1996b) in the oil, unlike in conventional refining where all the carotenoids are destroyed. Red palm olein is therefore the

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first physically refined vegetable oil rich in natural carotenoids and vitamin E. Another interesting and valuable component present in red palm olein is ubiquinone (Hamid *et al.*, 1995). The process for production of red palm olein has been commercialized and the product is currently being marketed locally and abroad. The purposes of this study are to obtain detailed information on the types and quantities of carotenoids, vitamin E, ubiquinones and sterols in the CRPo, and its fatty acids composition.

EXPERIMENTAL PROCEDURES

Materials

The CRPo used in this study was obtained from Carotino Sdn. Bhd. It bears the trade name Carotino (premium grade). For carotene analysis, the HPLC grade acetonitrile used was from J.T. Baker and the gradient grade methylene chloride was from Merck. For ubiquinone analysis, HPLC grade methanol and hexane were from Merck. For vitamin E analysis, HPLC grade hexane from Merck was used, tetrahydrofuran of gradient grade from Merck and AR grade isopropyl alcohol from Merck. Cholesterol, stigmasterol, campesterol, β-sitosterol and squalene standards were purchased from Sigma Chemical. Ubiquinone-10, used as an authentic standard in the study, was from Sigma Chemical. Petroleum ether (b.p. 40°C- 60°C) and ethanol used for saponification were of AR grade.

Preparation of Samples for Carotene, Sterol and Ubiquinone Analyses

The PORIM Test Method (1995) for saponification was used with modification. Approximately 5 g of the oil was saponified with 5 ml 50% ethanolic KOH by heating at 50°C in the dark in a water bath under a stream of nitrogen for 45 min. The saponified sample was then cooled to room temperature and extracted with 50 ml portions of petroleum ether until the supernatant became colourless. The pooled petroleum ether extracts were washed four times with 50 ml portions of distilled water and dried over anhydrous sodium sulphate. The extract was then dried in a rotary evaporator at 50°C. For carotene analysis, the residue was dissolved in a known volume of mobile phase for HPLC analysis.

HPLC Analysis of Carotenes

Chromatographic analyses of carotenes profiles were performed using a Waters 991-MS system with photodiode array detector (PDA). The PDA covered a spectral range of 190-799 nm. Detection was recorded at different wavelength maxima of the carotenoids. Carotenoids were identified based on their spectra and elution order as described by Ng and Tan (1988). Isocratic separation was performed on a Metaphase ODS C18 column (4.6 mm i.d. x 25 cm, stainless steel, 5 μm spherical particles; JASCO Corporation, Japan). A solvent system of acetonitrile (89%) and methylene chloride (11%) was used, at a flow rate of 1 ml min^{-1}.

Determination of Carotene Content

The carotene content was determined as described in PORIM Test Methods and expressed as β-carotene in parts per million (ppm).

HPLC Analysis of Ubiquinone

The oil samples were also subjected to saponification as described for carotene analysis. The unsaponifiable materials were subjected to separation by preparative thin-layer chromatography (TLC) as described by Hamid *et al.* (1995). HPLC analysis was also performed using a Waters 991-MS system with PDA detector, and Nucleosil ODS reverse phase C18 (RP18) column (4.6 mm x 25 cm) with the detector set at 190-350 nm range. The solvent system of methanol: hexane in 6:1 ratio was used, at a flow rate of 1 ml min^{-1}. Quantification was done by absolute calibration with pure standards.

HPLC Analysis of Tocopherols and Tocotrienols

Detailed analyses of the tocopherol and tocotrienol contents in various oil samples were carried out by normal phase HPLC with a (25 x 0.46 cm) Licosorp analytical column (Merck, Darmstadt, Germany), protected by a guard column (1.5 x 0.46 cm, 10 μm). A solvent

system of 94:5:1 (hexane/tetrahydrofuran/ isopropanol, vol/vol/vol) with a flow rate of 1.0 ml min^{-1} was applied, and the components detected with a scanning fluorescent detector set at wavelengths of 296 and 323 nm. Quantification was done by absolute calibration with pure standards.

Determination of Sterols

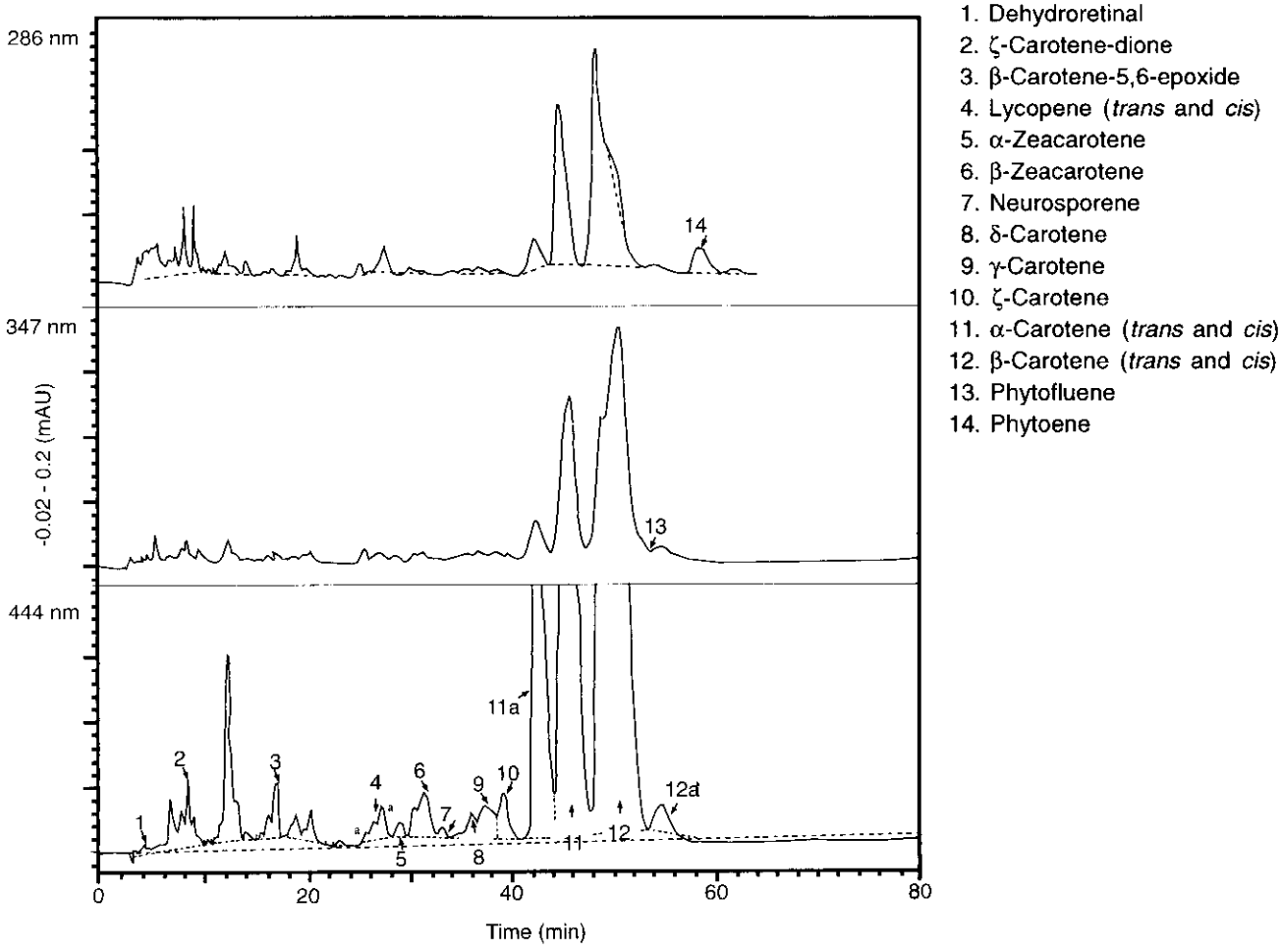
Sterols from the unsaponifiable matter were isolated by preparative TLC with a solvent system of chloroform/diethyl ether/acetic acid at 99:5:1 ratio (vol/vol/vol). The sterol band was identified by spraying with vanilin. It was recovered by extraction with chloroform and derivatized with N-O-*bis*(trimethylsilyl)-trifluoroacetamide reagent followed by analysis with a Hewlett Packard 5890 gas chromatography (15 m x 0.25 µm BPX5 column). Identification of the sterols was done by comparing with authentic samples. Squalene was added as an internal standard immediately prior to derivatization for quantitative determinations.

Fatty Acid Composition

The fatty acid compositions were determined according to ISO 5508: Animal and Vegetable Fat and oil analysis by gas-liquid chromatography of methyl esters of fatty acids.

RESULTS AND DISCUSSION

The HPLC chromatograms of carotenoid profiles of CRPo are shown in *Figure 1*. The carotene composition of CRPo and the UV absorption maxima of the various carotenes are shown in *Tables 1* and *2*, respectively. All the 11 caro-



1. Dehydroretinal
2. ζ-Carotene-dione
3. β-Carotene-5,6-epoxide
4. Lycopene (*trans* and *cis*)
5. α-Zeacarotene
6. β-Zeacarotene
7. Neurosporene
8. δ-Carotene
9. γ-Carotene
10. ζ-Carotene
11. α-Carotene (*trans* and *cis*)
12. β-Carotene (*trans* and *cis*)
13. Phytofluene
14. Phytoene

Figure 1. HPLC chromatogram of carotenoids of CRPo at wavelength (a) 286 nm (b) 347 nm (c) 444 nm (a denotes cis *isomers).*

TABLE 1. CAROTENE COMPOSITIONS (%) OF CRPo AND CPO (*tenera*)

Carotene	CRPo	CPO*
Phytoene	0.61 - 0.68	1.27
Phytofluene	0.15 - 0.17	0.06
β-Carotene	40.0 - 42.0	56.02
α-Carotene	40.6 - 41.9	35.06
Cis-α-carotene	9.0 - 11.4	2.49
ζ-Carotene	0.5 - 0.72	0.69
γ-Carotene	0.45 - 1.07	0.83
δ-Carotene	0.72 - 0.83	0.33
Neurosporene	0.11 - 0.26	0.29
β-Zeacarotene	1.17 - 1.33	0.74
α-Zeacarotene	0.50 - 0.56	0.23
Lycopene	0.86 - 1.07	1.30
Total (ppm)	665**	500-700

Note: ** Average of three samples done in duplicate.
Source: * Choo *et al.* (1996a).

TABLE 2. MAIN ABSORPTION MAXIMA (nm) OF CAROTENES

Carotene	This study				Davies, 1976		
Phytoene	276	287	299		276	286	297
Phytofluene	330	343	360		331	347	366
β-Carotene	430	444	480		425	450	477
α-Carotene	425	444	475		420	442	472
Cis-α-carotene	330	415	438	470		-	
ζ-Carotene	383	406	420		380	401	426
γ-Carotene	436	461	488		437	462	492
δ-Carotene	431	459	488		428	458	490
Neurosporene	*cis*	335	416	443 467	416	440	470
β-Zeacarotene	404	426	452		407	427	454
α-Zeacarotene	401	424	449		398	421	449
Lycopene	*trans*-	446	470	503 448	473	504	
	cis-	349	438	467 488			

tenes in CPO were preserved in CRPo. This shows the efficiency of the new process, whereby even the minor carotenoids were largely intact despite the vulnerability of carotenoids towards heat. This is probably due to the mild conditions adopted in the deodorization and deacidification of the oil.

The α- and β-carotenes, including their *cis* isomers, constitute more than 80% of the carotenes in CRPo. The carotene profile of CPO showed a higher content of β-carotene than α-carotene, whereas they are almost equal in CRPo. Both these carotenes possess provitamin A activity. Vitamin A equivalents of CRPo and the recommended daily allowances (RDA) are shown in *Table 3.*

TABLE 3. VITAMIN A EQUIVALENT OF CRPo AND RDA

One retinol equivalent (RE) = 1 μg of retinol = 6 μg of β-carotene = 12 μg of other provitamin A carotenes (Tee, 1992).

Carotene content of CRPo
(ppm or μg g^{-1} β-carotene) = 665 μg g^{-1}
Average composition of carotenes:
α-Carotene = 41%
β-Carotene = 41%
γ-Carotene = 0.76%
β-Zeacarotene = 1.25

Vitamin A equivalents of CRPo with 665 ppm of carotenes: about 92 μg RE g^{-1} oil.

Amount per day for vitamin A (plus carotenoids):
Children 1 to 10 years, 400-700 μg RE.
Males 11- > 51 years, 1000 μg RE.
Females 11- > 51 years, 800 μg RE.
Pregnant women, +200 μg RE.
Lactating women, +400 μg RE.

Source: RDA, Ninth edition (revised 1980).

CRPo is a good and safe source of vitamin A, as there is no risk from vitamin A overdose. β-Carotene is absorbed from the intestine with less efficiency as the dietary intake of β-carotene increases. The conversion of β-carotene to vitamin A also declines with increasing β-carotene intake (Goodwin, 1984). The consequence is an increase in the β-carotene levels circulating in the blood with no significant increase in circulating level of vitamin A. Other minor carotenes such as γ-carotene and β-zeacarotene also possess vitamin A activity (Choo, 1995).

Aside from provitamin A activity, the carotenes such as β-carotene,α-carotene and lycopene are effective antioxidants and singlet oxygen quenchers (Dimascio *et al.,* 1989). A study by Serbinova *et al.* (1992) using palm-based carotenes showed that the order of strength in *in vitro* lipid peroxidation inhibition was α-carotene > lycopene > β-carotene. The antioxidant property explains the importance of β-carotene

as there are considerable evidence from epidemiological and animal data linking β-carotene and other carotenoids to decreased risk of some cancers (Ziegler, 1989; Ziegler *et al.*, 1996a,b). Other interesting findings are that α-carotene is 10 times more potent as an anti-cancer agent than β-carotene (Murakoshi *et al.;* 1989; 1992). β-Carotene is also reported to have a positive effect in the reduction of atherosclerotic plaque in the arteries (Gaziano *et al.*, 1990). Manorama *et al.* (1993) showed that red palm oil (rpo) effectively prevented chemical carcinogenesis in rats more than refined bleached deodorized olein (rbdo), rbd palm olein, attributing the effectiveness of rpo to its carotenoids. The *in vivo* and *in vitro* chemical carcinogenesis studies by Tan and Chu (1991) in the rat hepatic cytochrome-P450-mediated monoxygenase system showed the order of anti-tumour reactivity was palm oil (with carotenoids) > beta carotene > canthaxanthin > palm oil (without carotenoids). With a myriad of medicinal properties associated with carotenes, it is indeed gratifying to find that the PORIM process is able to retain such a high concentration of carotenoids (approximately 600 ppm) in CRPo.

Xanthophylls

Dehydroretinal, ζ-caroten-dione and β-carotene-5,6-epoxide isomers were also tentatively identified by comparison of the spectra and their elution order as described by Ng and Tan (1988). The UV spectra of these xanthophylls are shown in *Figures 2a,b* and *c*. No important physiological effect has been attributed to these xanthophylls to date. However, Terao (1989) found that carotenoids with *oxo* groups are more effective antioxidants than β-carotene against peroxyl radical attack on lipids. They suggested that the presence of oxygen atoms reduces the unpaired electron density on the carbon skeleton and enhances the reactivity of the carbon-centred radical in trapping molecular oxygen. If this is the case, then the presence of *oxo* groups in ζ-caroten-dione may increase its effectiveness as antioxidant compared to the carotenes.

Vitamin E

The four major types of vitamin E (α-

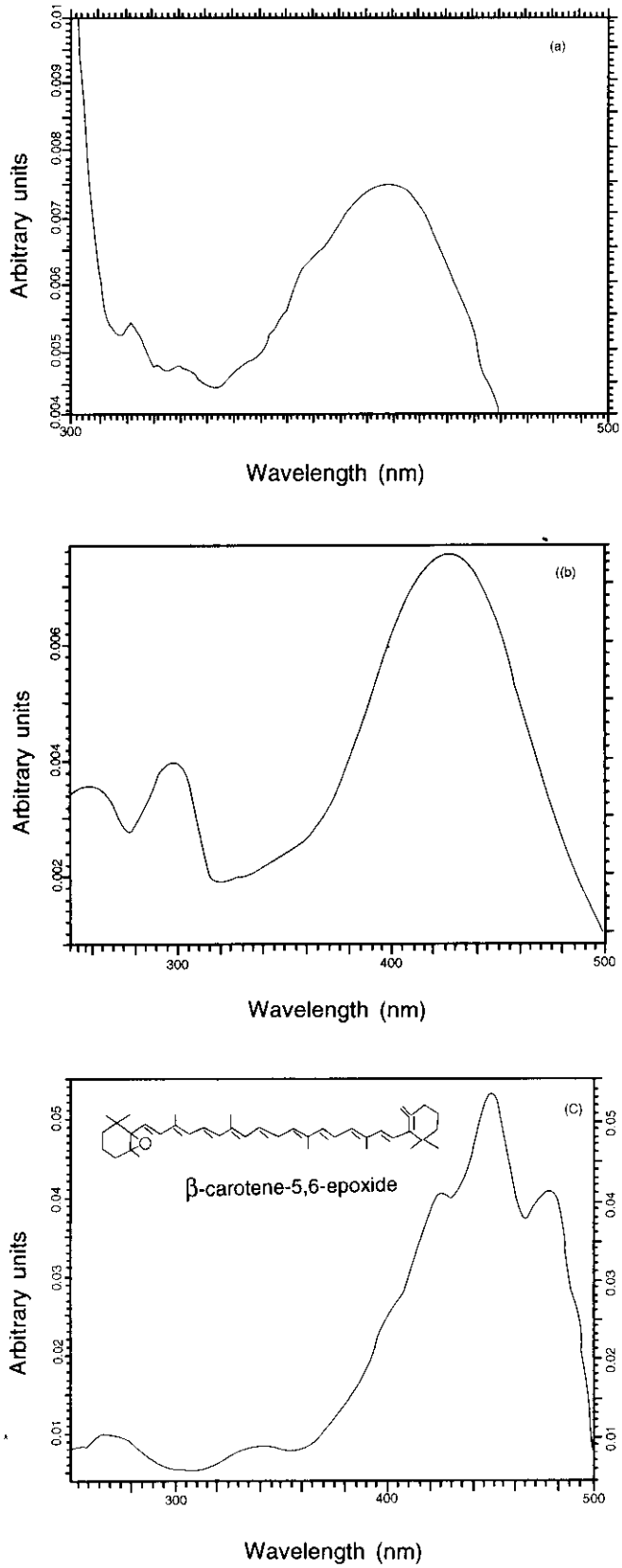


Figure 2. UV spectra of xanthophylls of sample (a) dehydroretinal (b) ζ-caroten-dione (c) β-carotene-5,6-epoxide.

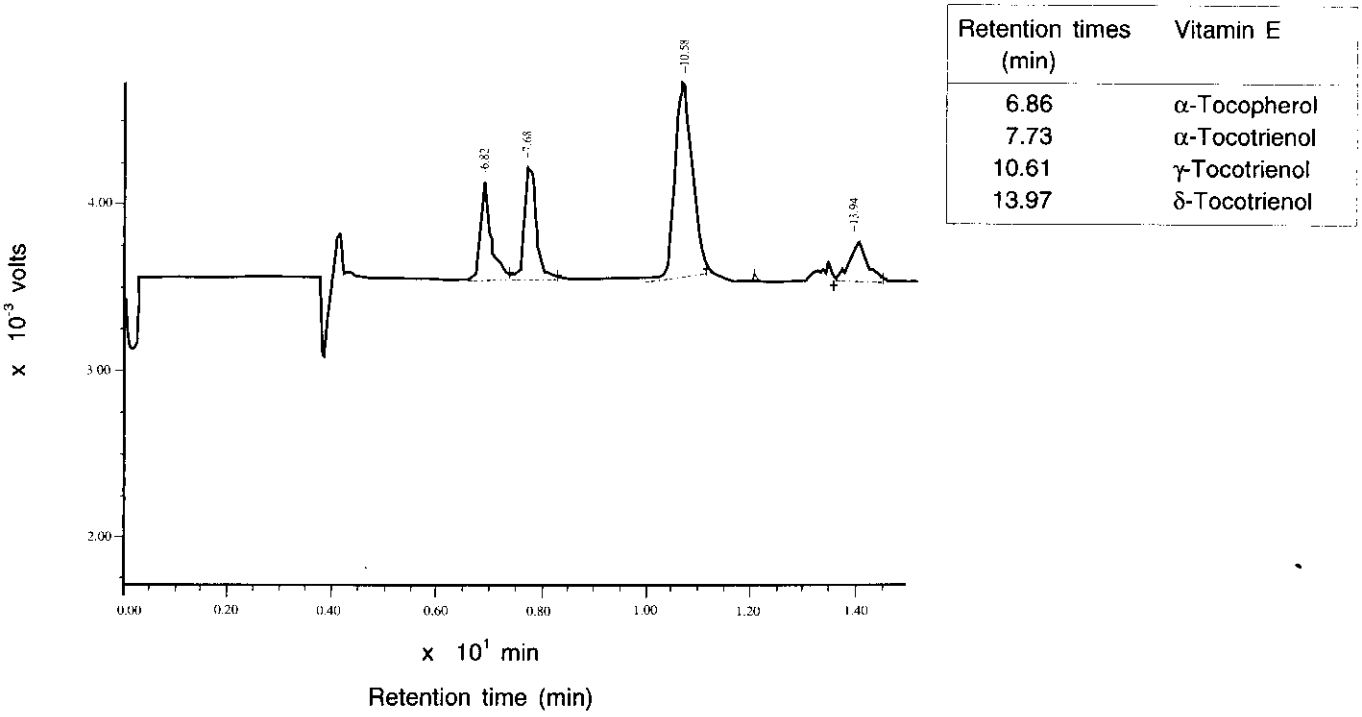


Figure 3. HPLC chromatogram of vitamin E CRPo.

tocopherol, α-, γ-, and δ-tocotrienols) in CPO are preserved in CRPo as shown in *Figure 3*. Most of the vitamin E in CRPo are isomers of tocotrienols which constitute 80% of the total vitamin E. The composition of the vitamin E components is also comparable to that of CPO (*Table 4*).

The concentration of vitamin E in CRPo is 717-863 ppm with an average of 810 ppm was much higher than the rbdo level of 515-800 ppm. This is understandable because the conventional method of refining CPO only retains 70% of the vitamin E in the refined palm oil. The rest is distilled off together with the fatty acids (Gapor, 1983). The new process is therefore more efficient in retaining vitamin E in the oil. The USA National Research Council (NRC) (1989) has stipulated, for dietary purposes, that vitamin E activity be expressed as RRR-α-tocopherol equivalent (α-TE) where one α-TE is equivalent to 1 mg of RRR-α-tocopherol. The vitamin E equivalent of CRPo and the RDA (1980) are shown in *Table 5*.

The high vitamin E concentration of CRPo is beneficial because it stabilizes the oil, besides providing more protection for the carotenes and

TABLE 4. VITAMIN E COMPOSITIONS (%) AND CONTENTS OF CPO, CRPo AND rbdo

Samples	Vitamin E (%)				Vitamin E content (ppm)
	α-Tocopherol	α-Tocotrienol	γ-Tocotrienol	δ-Tocotrienol	
CPO**	21	24	43	11	600-1000
CRPo*	19	29	41	10	717-863
rbdo***	25	29	36	10	515-800

Note: *CRPo from two production batches analysed in duplicates.
Sources: **Choo *et al.* (1996a).
***Gapor (1990).

TABLE 5. VITAMIN E EQUIVALENT OF CRPo AND RDA FOR VITAMIN E

One α-TE = 1 mg of RRR-α-tocopherol = 1.49 IU (American Institute of Nutritition, 1979).

Vitamin E equivalent of 810 ppm of CRPo = 11.34 mg α-TE or 16.90 IU per 14 g oil (14 g is equivalent to one teaspoon oil).

Food and Nutrition Board, NRC (1989).
Adult males 10 mg α-TE or 15 IU.
Adult females 8 mg α-TE or 12 IU.

TABLE 6. FATTY ACID COMPOSITONS (%) OF CRPO, CPO AND rbdo

Fatty acid	CPO	CRPo*	rbdo
C6:0	-	-	-
C8:0	-	-	-
C10:0	-	-	-
C12:0	0.20	0.25	0.27
C14:0	1.10	1.07	1.09
C16:0	44.00	36.60	40.93
C16:1	0.10	-	-
C18:0	4.50	3.70	4.18
C18:1	39.20	46.70	41.51
C18:2	10.10	12.80	11.64
C18:3	0.40	-	0.40
C20:0	0.40	-	0.37

Note: *Samples from two production batch in duplicates.

unsaturated triglycerides against oxidative deterioration. In the case of carotene, it will ensure that they are not degraded to other forms which may result in the loss of important physiological properties. Recent studies (Haila and Marina, 1994; Haila *et al.*, 1996) showed a synergistic effect between the components of vitamin E and carotenoids in retarding auto- and photo-oxidation of the unsaturated triglycerides. They found the combined effects of carotenoids and vitamin E much more effective than their individual effects. Therefore, the rationale of retaining carotenoids with vitamin E in this new process is a correct one in the light of these recent findings.

Recent studies showed that tocotrienols of palm oil exhibit anti-cancer properties (Komiyama *et al.*, 1989; Guthrie *et al.*, 1993; Goh *et al.*, 1994; Nesaretnam *et al.*, 1992). Tocotrienols also exhibit greater physiological efficiency in inhibiting the growth of human and mouse tumour cells than tocopherols (Kato *et al.*, 1985; Komiyama *et al.*, 1989; Sundram *et al.*, 1989). Kamar *et al.* (1997) showed that tocotrienols from palm olein effectively inhibited protein and lipid peroxidation in rat liver microsomes suggesting that palm oil tocotrienols are effective natural antioxidants capable of protecting celullar membranes against oxidative damage.

Fatty Acid Composition

CRPo (*Table 6*) has 46.7% C18:1 much higher than normal rbdo (41.51%). Monounsaturated fats have been reported effective in lowering blood cholesterol (Mattson and Grundy, 1985). The total unsaturated fats in CRPo is 58%. The major saturated fatty acids are mainly palmitic (C16:0) 36.6% and stearic acid (C18:0) 3.7%. Nevertheless, their contents are lower compared to rbdo which has 40.89% palmitic and 4.18% stearic acid. Unlike myristic acid, these two saturated fatty acids have not been found to be harmful to human health. Both stearic and palmitic acids do not raise blood cholesterol in people who are normocholesterolemic (Hayes, 1993; Khosla and Hayes, 1992; 1994; Hayes *et al.*, 1995; 1991). Therefore, with the hypocholesterolemic effect of its monounsaturated fatty acid and the neutral effect of its major saturated fatty acids, CRPo can be consumed with no health risk.

Sterols

All the major sterols in CPO are retained in CRPo as shown by its compositional profile (*Table 7*), with β-sitosterol at 59%, campesterol 22% and stigmasterol 17%. Cholesterol and other minor sterols are present at very small levels (< 2.6%). Sterols are important because they have great potential in the pharmaceutical industry when converted into steroid derivatives (Hedtmann *et al.*, 1988). The high content of β-sitosterol in CRPo is beneficial because it is known to be hypocholesterolemic (Farquhar, 1996). The sterol content of CRPo is higher than rbdo. This shows that this newly developed process is more efficient and only results in slight loss of sterols as compared to conventional refining.

TABLE 7. STEROL COMPONENTS (ppm) OF CPO, rbdo AND CRPo

Sample	Cholesterol	Campesterol	Stigmasterol	β-Sitosterol	Unknown	Total/ppm
CPO*	7 - 13	90 - 151	44 - 66	218 - 370	2 - 18	326-527
Rbdo**	2.1 - 2.4	25.6 - 30.4	12.4-23.3	67.7 - 114	Nil - 1.2	109-170
CRPo	6.6 - 11.5	76 - 83	59 - 64	187 - 218	< 6	325-365

Sources: *Rossell *et al.* (1983).
**Siew (1990).

Ubiquinone Profiles

CPO contains 10-80 ppm ubiquinone-10 (UQ10) and about 5 ppm of ubiquinone-9 (UQ9) (Hamid *et al.*, 1995). Only the UQ10 was detected in CRPo with a concentration of 18 to 25 ppm. Virtually no UQ9 was detected in CRPo, this is understandable as the starting concentration was already low. As UQs are sensitive to alkaline, acidic and high temperature conditions, they would decrease during processing. Many therapeutic values of UQ10 have been reported, such as enhancement of the immune system (Lenaz, 1985), prevention of heart diseases and hypertension (Yamamura, 1985). The UQ10 has also demonstrated antioxidative activity *in vitro* (Mellors and Tappel, 1966) and in intact animals (Beyer, 1989). Zamora *et al.* (1991) showed that lipid peroxidation and damage to red blood cells were prevented in a manner similar to that by vitamin E and selenium.

CONCLUSION

All the valuable minor components of CPO such as carotenoids, vitamin E, sterols and ubiquinones were successfully retained in CRPo produced by molecular distillation. The virtue of CRPo is its high concentrations of carotenoids, vitamin E isomers and sterols compared to conventional rbdo. Therefore, CRPo is highly nutritious.

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