ABSTRACT

A total of 11 types of carotenes and 7 *cis* isomers present in the commercial crude palm oil have been identified using non-aqueous, reversed-phase liquid chromatography (NARP-HPLC); the major carotenes present are α- and β-carotenes and other carotenes are phytoene, phytofluene, ζ-carotene, δ-carotene, γ-carotene, neurosporene, α-zeacarotene, β-zeacarotene and lycopene. The analytical technique has been developed for the routine determination of the carotene profiles in the oils from various oil palm species/varieties and palm oil products.

Although the total carotene contents varied significantly among the varieties of *Elaeis guineensis* (including Dura, Pisifera and Tenera), *E. oleifera* species and hybrids of *E. guineensis* and *E. oleifera*, the carotene profiles of the oils from these oil palm varieties/species and hybrids are similar. The total amount of α- and β-carotenes constitutes more than 87% of the total carotene contents, with α-carotene and β-carotene ranging from 24 to 40% and 54 to 61% respectively. However, the minor carotene component, lycopene, was found to be relatively higher (more than 17 times) in all the *E. guineensis* varieties as compared to the *E. oleifera* and its hybrids. High levels of lycopene in the exocarp impart a dark red colour to the fruits of *E. guineensis* varieties when they are ripe, but fruits of *E. oleifera* and its hybrids remain orange in colour. Analyses on vitamin E and sterol compositions by high-performance liquid chromatography and gas chromatography respectively, showed no significant differences among the various oil palm species/varieties and the hybrids.

Carotene-enriched oils have been obtained from pressed palm-fruit fibres and second-pressings of the mesocarp, the carotene contents of these oils being 6-8 and 3-4 times higher, respectively, than commercial crude palm oil. Detail carotene analyses showed that α- and β-carotenes are still the major carotenes in these oils, but their relative amounts (*ca.* 20

and 30% of the total carotenes respectively) were lower than those of crude palm oil. Other carotenes such as lycopene, ζ-carotene and phytoene were found to be in relatively higher quantities. It was found that the higher carotene concentrations in these oils were mainly due to the high carotene content in the exocarp, the carotenes of which were not totally extracted by the conventional single screw-press in the palm oil mill. The oil extracted from the exocarp of oil palm fruits has been found to contain a higher carotene concentration (> 5700 ppm), the carotene profile being similar to the oils extracted from pressed fibres and from a second-pressings of the mesocarp. The high lycopene content in the exocarp (13% of the total carotenes) again causes the skin of the oil palm fruits (*E. guineensis* varieties) to appear dark red in colour.

Vitamin E and sterol contents were also found to be high in the oils from pressed fibres and second-pressings of the mesocarp; this indicated that a substantial amount of vitamin E and sterols still remain in the fibre after the conventional oil extraction. Among the vitamin E components, α-tocopherol was found to be the major component (46-67%) present in the oils from fibres, second-pressed mesocarp and the exocarp, indicating that the vitamin E profile of the exocarp is slightly different from the mesocarp (for commercial palm oil), which normally provides about 21% α-tocopherol with the rest being mainly tocotrienols. No differences were observed in the sterol compositions of oils from the various varieties and hybrids.

Extraction of oil from pressed palm-fruit fibres was also carried out in a high pressure Soxhlet extractor using liquid CO_2. The oil could be extracted almost completely within 2 hours at a pressure of 750 psi and heating temperature of 45°C. Fibre oil extracted using this method was of good quality as it had relatively higher vitamin E but lower phosphorus contents than commercial crude oil.

Two processes have been successfully developed to recover or retain the carotenes in crude palm oil which are presently destroyed during the refining process. The first method involved transesterification of oil to alkyl esters, followed by a molecular distillation of the

esters. This process can produce a concentrate with a carotene concentration of up to 40,000 ppm at a recovery of more than 80%. The carotene profile remains similar to that of the original crude palm oil. A presentation of the carotene concentrate in powder form has been successfully made and its storage stability was acceptable. The carotene concentrate is also marketable as a triglyceride paste, powder or capsules, and these forms are useful for pharmaceutical purposes or as food colourants in addition to providing vitamin A. The second method involved a two-stage process to refine crude palm oil but yet retain the carotenes. This process has been scaled up to a pilot plant scale. The first stage involves a mild pretreatment of crude palm oil with phosphoric acid and bleaching earth to remove phospholipids, impurities and some oxidative products. The second stage is a low temperature (< 165°C) deodorisation by a molecular distillation unit under high vacuum (< 20 mtorr). This removed the odorous compounds and reduced the fatty acid content to less than 0.1%, but allowed almost all the carotenes and more than 80% of vitamin E to be retained. After refining, the red palm oil had comparable quality parameters with commercial refined, bleached and deodorised palm oil. Red palm oil also showed an oxidative stability similar to refined palm oil based on Rancimate tests and was also of good quality according to sensory evaluation. This product was found to be of nutritional value for various food applications such as cooking oil for various local dishes, salad dressing, cake making, margarine and ice-cream formulations.

An *in vitro* study on the photoprotective and antioxidant activities of palm carotenes has revealed that palm carotenes can be an effective photoprotective agent in the photosensitised oxidation of palm methyl esters and low density lipoprotein (LDL) samples. However, no significant protective effect was shown by palm carotenes in the Cu^{2+}-induced autoxidation of LDL.

Antioxidant activities of palm carotenes, together with vitamin E, ascorbic acid and dihydrolipoic acid, were also investigated in the 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH)-induced oxidations of LDL and plasma. No significant protective effect by palm

carotenes was confirmed. Hydrophilic antioxidants, *viz.* ascorbic and dihydrolipoic acids, were found to be very effective in protecting against lipid oxidation in LDL. In LDL samples supplemented with these acids, the endogenous vitamin E concentrations remained high and lipid peroxides were undetectable at the end of the oxidation experiments. In comparison, LDL controls (without any added exogenous antioxidants) and LDL samples with carotene supplementation were found to give rise to lipid peroxides once the endogenous vitamin E was completely consumed. In the AAPH-induced oxidation of plasma, endogenous vitamin E was found to be ineffective in protecting the lipid and protein peroxidation in plasma and oxidation was found to take place even though the endogenous vitamin E was still present at relatively high levels. Ascorbic and dihydrolipoic acids, on the other hand, were very effective in protecting against peroxidation of plasma proteins and lipids induced by radicals originating from the aqueous phase.

In the study of palm carotene distribution in animal plasma and organs, NARP-HPLC was employed and a photodiode array detector was used to determine the carotene profile as well as the concentrations of retinol and retinyl esters present. Rabbits fed with diets enriched with palm carotenes were found to store various carotenes in the plasma and organ tissues in variable amounts, especially in the liver, spleen and adrenal glands. The major carotenes detected were phytoene, phytofluene and ζ-carotene, with almost all α- and β-carotenes being metabolised to retinol and retinyl esters. Only small amounts of carotenes remained in the rabbit's plasma and organ tissues but large quantities of retinol and retinyl esters were detected in the liver and to a lesser extent, the pancreas. The content of retinol and retinyl esters in the liver (diet supplemented with palm carotenes) were 3 and 30 times higher respectively than in the rabbit groups fed diets without palm carotenes. The major retinyl esters present in the organ tissues were retinyl palmitate, oleate and linoleate, whereas only retinyl palmitate and stearate were found to be present in predominant quantities in the plasma. α-Retinyl esters, which were metabolised from α-carotene (or ε,β-carotene), were also found to be present in

the liver and pancreas. Relatively small amounts of carotenes were detectable in the plasma, liver and spleen in rabbits fed with diets enriched with palm carotenes but depleted of palm vitamin E (< 10 ppm). As expected, retinyl esters were detected at low levels in plasma, liver and pancreas of rabbits fed with diets without carotenes. These results showed that vitamin E, as an antioxidant, exerts a sparing (protective) action on carotenes and their metabolites in the diet.

Oxidative susceptibilities of LDL isolated from rabbits fed with palm olein (with or without supplementation of palm carotenes and/or palm vitamin E) were compared to those of other dietary fat groups including soybean and coconut oils. Different dietary fats were found to affect (but not drastically) the fatty acid compositions of plasma and LDL. More linoleic and oleic acids were found in the plasma and LDL of the rabbits fed with soybean and palm olein diets respectively, and relatively higher levels of lauric and myristic acids were found in those fed with coconut oil diet. Oxidative susceptibilities of LDL from various dietary groups were investigated based on the Cu^{2+}-induced oxidation, from which lag times (or induction periods), lag phase gradients and propagation phase gradients were available from the kinetic plots of conjugated-diene formation. LDL samples from all the rabbits fed with palm olein were found to have longer lag times (i.e. most stable to oxidation) as compared to other dietary fat (soya and coconut oils) groups. However, no significant difference was observed in the lag times of LDL samples from animals fed with palm olein diets (with or without supplement of palm carotenes), although a lower propagation phase gradient was observed in the palm carotene-supplemented groups. Highest propagation phase gradients and shortest lag times (low stability) were shown by the LDL from soybean-diets due to a combination of higher polyunsaturated fatty acids (great demand for protection by vitamin E and other antioxidants) and lower plasma vitamin E levels.