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Effect of Processing on β-Carotene Retention in Crude Palm Oil and its Products

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ABSTRACT

*Crude palm oil (*Elaeis guineensis*) can serve as a promising source of β-carotene in developing countries where vitamin A deficiency is prevalent, apart from fulfilling other functions of an edible oil. Non-aqueous reverse phase HPLC analysis with a UV–Vis detector at 450 nm was carried out to estimate the amount of β-carotene in crude palm oil (CPO) produced in India. Isocratic elution with acetonitrile/methanol/dichloromethane (60%/35%/5%) at 1·5 ml/min on a 25 cm C_{18} column eluted β-carotene at 14·5 min. β-Carotene content was estimated to be around 370 ppm, which amounts to about 70% of the total carotenoids (540 ppm—estimated spectrophotometrically at 450 nm $E_{1\,cm}1\% = 2500$). Sensitivity and accuracy of the method was observed to be high as indicated by the standard graph of β-carotene and recovery of standard added to sample (100%).*

Effect of different cooking methods like baking, seasoning, deep frying and shallow frying on retention of β-carotene was studied and it was observed that 70–88% of it was retained in the cooked foods. Repeated deep frying, using the oil five times consecutively, resulted in a total loss of β-carotene by the fourth frying stage itself and alteration of its organoleptic, physical and chemical properties.

Hence, CPO may be suitable for single frying operations only or for preparations which involve a short heating time and completely take up the oil into the cooked product, e.g. 'Cake', 'Upma', 'Kichidi', 'Suji halwa'. Probably 'Suji halwa' can be selected as an ideal choice for vitamin A supplementation to vulnerable children because it is well accepted, works out to be cheap per RDA serving and it retains 70% of its β-carotene after cooking.

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INTRODUCTION

β-Carotene is a member of the family of carotenoids, which are pigments found in all photosynthesizing organisms including plants and bacteria. Of the approximately 50 carotenoids with pro-vitamin A activity, β-carotene is the carotenoid with the greatest pro-vitamin A biological activity (Freed, 1966). Recent evidence also suggests that such carotenoids, in addition to their capability to act as provitamin A precursors, may be involved in other biochemical processes; for example, β-carotene can serve as an anti-oxidant (Burton & Ingold, 1984) as well as a potent quencher of the highly reactive molecular species, singlet oxygen (Foot *et al.*, 1970). It's role as a protective agent against cancer has been postulated (Peto *et al.*, 1981).

In many developing countries, vitamin A deficiency occurs among children during the weaning period, which constitutes a major public health problem. India is also one of these countries where vitamin A prophylaxis programmes are being carried out on a massive scale (Vijayaraghavan *et al.*, 1984). Therefore, the search for new sources of β-carotene for use in supplementary feeding programmes, coupled with a severe edible oil shortage in India during 1986–88, had led to the consideration of the possibility of using crude palm oil (*Elaeis guineensis*) (CPO) as an edible oil. CPO has been reported to be a very rich source of β-carotene (Maclellan, 1983).

Oil Palm India Limited, under the Technology Mission for Oilseeds, has launched a massive oil palm cultivation programme in many parts of South India with the objective of making India self-sufficient in edible oils (Abraham, 1988). The Council of Scientific and Industrial Research Laboratories at Trivandrum, India, have developed the technology for the production of edible grade CPO and provided the sample for all our investigations.

Preliminary nutritional and toxicological evaluation of CPO has been completed and it was found to be nutritionally adequate and toxicologically safe for human consumption (Manorama & Rukmini, 1989; Manorama *et al.*, 1989).

To evaluate the possibility of using CPO in supplementary feeding programmes for pre-school and school-going children, two aspects need to be investigated, (a) β-carotene retention in CPO during different methods of cooking and stability during repeated deep frying, (b) efficiency of absorption of β-carotene in children. In this paper, loss of β-carotene in different cooked products has been reported. The objective of this study was to determine the amount of β-carotene that can be supplied in the form of different cooked recipes to children in feeding programmes.

MATERIALS AND METHODS

Materials

The sample of edible grade CPO was obtained from the Regional Research Laboratories of the Council of Scientific and Industrial Research situated at Trivandrum, Kerala, India.

Standard β-carotene (crystalline, obtained from carrots) was obtained from Sigma Chemical Company, St Louis, USA, (Product No. C-0126). HPLC grade acetonitrile, methanol and methylene chloride were obtained from Spectrochem (Bombay, India). Petroleum ether 60–80° used for extraction was of superior analytical grade. Butylated hydroxy toluene was obtained from Sigma. The HPLC system used was a Shimadzu LC-6A model fitted with an automatic degassing unit; a system controller, model SCL6A; Shimadzu UV–Vis spectrophotometric detector, model SPD-6AV; pumps, model LC-6A; rheodyne injection valve with 20 μl loop; Shodex column ODS C18 F-411A, 25 cm × 4·6 mm and 5 μm particle size; integrator, model C-R3A-Chromatopac.

Experimental

Cooking losses

A number of recipes were developed using CPO, out of which four common preparations were selected for analysis of their β-carotene content. Selection was made in order to have a range of different types of heat treatments used during food preparation. The four recipes selected were as follows:

(a) *Upma:* A breakfast item commonly consumed in South India, made essentially with semolina, using CPO for seasoning with different spices (cooking temperature 180 ± 3°C).
(b) *Cake:* An oven-baked (220°C) batter of flour, eggs and sugar using CPO as the shortening medium.
(c) *Muruku:* A deep-fried extruded snack made of chick pea flour and rice flour (2:1), where CPO was used as the frying medium (frying temperature 180 ± 3°C).
(d) *Suji halwa:* A sweet preparation made with semolina, where CPO was used as a shallow frying medium (cooking temperature 180 ± 3°C).

Losses due to repeated frying

CPO was heated up to 180 ± 3°C and potato chips were fried till they were done to a golden brown colour for approximately 10 min. The oil was cooled

for 1 h and again heated up to 180 ± 3°C and a second batch of chips were fried. The procedure was repeated three more times. At the end of each frying, an aliquot of CPO was removed and stored in the dark at −20°C for analysis of β-carotene.

Processing of samples for HPLC separation

CPO was saponified to extract the β-carotene completely before estimation by the method standardized by Ng and Tan (1988). CPO (2–3 g) or 10 g of homogenized food material was dissolved in 20 ml of dichloromethane, in a 250 ml flask with side outlet and 3 ml of 1% (w/v) Butylated hydroxy toluene (BHT), in methanol and 45 ml of 17% (w/v) potassium hydroxide in methanol were added. The contents of the flask were stirred on a magnetic stirrer for 3 h at room temperature with a constant flow of nitrogen in a dark room.

The saponified mixture was extracted with 25-ml portions of petroleum ether 60–80°, until a clear white colourless extract was obtained. Precipitated soaps can either be filtered through a Buchner funnel under vacuum after thorough washing or centrifuged twice or three times adding petroleum ether to ensure complete extraction of carotenoids.

The pooled petroleum ether layer was washed with four 50-ml portions of 8% (1·5M) aqueous sodium chloride followed by four 50-ml portions of distilled water. The washed solvent was passed through a column packed with sodium sulphate to remove moisture.

The volume of petroleum ether was accurately measured and the OD read in a visible Gilford spectrophotometer at 450 nm and the concentration of total carotenoids determined using the molar extinction coefficient $E_{1\,cm}$ 1% = 2500.

The solvent was concentrated to about 5 ml by rotary evaporation with a water bath temperature of 27°C. The concentrated extract was stored overnight at −20°C and centrifuged for precipitation of sterols. The supernatant was carefully withdrawn, evaporated to dryness under nitrogen and redissolved in dichloromethane and stored under nitrogen at −20°C.

Standardization and HPLC separation

A mobile phase of acetonitrole/methanol/dichloromethane (60%/35%/5%) was used in this experiment.

Standard β-carotene was dissolved in petroleum ether, evaporated under nitrogen and redissolved in dichloromethane. Standard concentration was determined spectrophotometrically using a molar extinction coefficient of $E_{1\,cm}$ 1% 2530 (specified by Sigma for the batch) and different concentrations

were prepared for HPLC analysis and to plot the standard graph. A correlation coefficient was calculated to assess linearity between concentration of standard and peak area.

Samples were diluted suitably to fall within the standard range for injection and separation. Analysis was conducted at a solvent flow rate of 1·5 ml/min, with a detector sensitivity (AUFS) of 0·02 and wavelength of 450 nm. β-Carotene concentration was calculated both from standard graph and also using the formula.

$$\mu g/g\ \beta\text{-carotene} = \frac{\text{Peak area of sample}}{\text{Peak area of standard}} \times \text{conc. of standard} \times \text{dilution factor}$$

where a known concentration of standard is injected along with each batch of samples.

RESULTS AND DISCUSSION

The major objective of this experiment was to identify a suitable method for efficient extraction and estimation of β-carotene in CPO and to apply this method for finding out the quantity of β-carotene available for human consumption in the cooked preparations, as it is widely known to be unstable to heat and oxidation (Freed, 1966; Okiy & Oke, 1986). A number of studies have been published describing the extraction and quantitative estimation of β-carotene in vegetables (Speek *et al.*, 1986), serum (Katrangi *et al.*, 1984; Aaran & Nikkari, 1988) and algae (Nells & De Leenher, 1983). However, it is more difficult to extract carotenoids present in an edible oil, because they are the constituents of the unsaponifiable matter of the oil and hence, have to be effectively extracted into an organic solvent only after thorough saponification has been done. For extraction of carotenoids from vegetables, thorough homogenization of the plant material with the appropriate solvent is sufficient to ensure complete extraction of carotenoids (Nells & De Leenher, 1983). In a recent study on analysis of palm oil carotenoids (Ng & Tan, 1988), conditions for extraction were standardized and discussed. The same procedure was followed in the present study with minor modifications to ensure thorough removal of carotenoids from soaps.

Non-aqueous reverse phase (NARP) HPLC analysis was reported to be superior to other conventional reverse phase chromatography for separation of carotenoids because of its ability to chromatograph both polar and non-polar derivatives isocratically (Nells & De Leenher, 1983). They observed that a typical NARP eluent consists of a polar component like acetonitrile, a non-polar modifier like dichloromethane to adjust solvent

strength and act as a good solubilizer for carotenoids and a small amount of methanol to optimize selectivity. The proportions of the three solvents they used were 70/10/20 of acetonitrile/methanol/dichloromethane. However, in another study (Ng & Tan, 1988) it was found that very poor resolution was obtained between α and β-carotene with 20% dichloromethane, hence the mobile phase was modified by decreasing the dichloromethane concentration to 5%, increasing methanol to 35% and decreasing acetonitrile to 60%.

Therefore, in the present study, the above proportion of solvents were used and all standards and samples were dissolved in dichloromethane for injection onto the HPLC system. Good resolution of β-carotene was obtained at a retention time of 14·5 min with a flow rate of 1·5 ml/min and 9·7 min at a flow rate of 2 ml/min.

Figure 1 represents the standard graph obtained by plotting different concentrations of standards versus peak area in arbitrary units. There was found to be a perfect positive correlation between concentration of standard β-carotene and peak area ($r = 0{\cdot}99$).

Due to non-availability of internal standards, a known concentration of standard was also injected along with each batch of samples for better precision and accuracy in estimation. Spiking of sample with a known amount of standard was also done to ensure that the peak identification according to retention time was accurate.

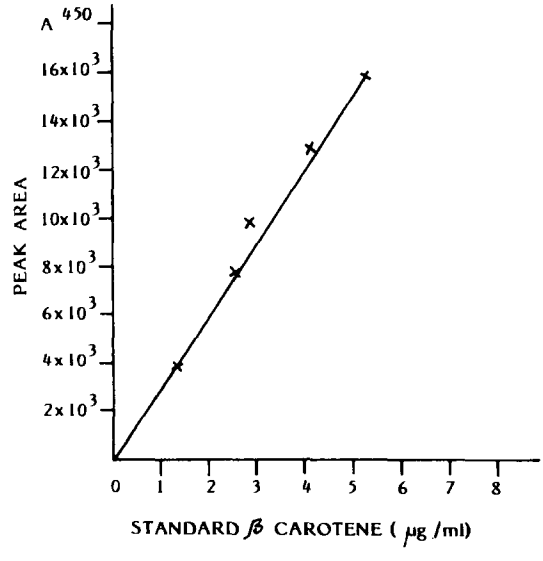

Fig. 1. Standard graph of different concentrations of β-carotene and the corresponding peak areas, eluted in 14·5 min with a mobile phase of acetonitrile/methanol/dichloromethane (60%/35%/5%) at a flow rate of 1·5 ml/min.

TABLE 1
Total and β-Carotene Contents of Crude Palm Oil

Total carotenoids (μg/g)	540·8
β-carotene (μg/g)	
Calculated from standard curve	370·1
Calculated from formula[a]	367·7
β-carotene as a percentage of total carotenes	70
Recovery of standard added to sample (%)	100

[a] As described in materials and methods.

Table 1 gives the total and β-carotene contents of CPO. Total carotenoids were found to be 540 μg/g and β-carotene was 370 μg/g, which is approximately 70% of total carotenoids. There was no difference found between β-carotene values calculated from the standard graph and using the formula mentioned. Ng and Tan (1988) have reported the β-carotene content of CPO (of Malaysian origin) to be 343 μg/g after 4 h saponification. Total carotenoids were reported to range from 500 to 700 ppm, 62% being β-carotene (Maclellan, 1983). The results of the present study are therefore, comparable with reported values. Spiking of samples with known amounts of standards resulted in 100% recovery of standard denoted by a corresponding increase in peak area. Figures 2a and 2b depict the elution profile of standard β-carotene and CPO carotenoids respectively. Our interest in this study was to quantitate only β-carotene and no attempt was made to identify and quantify other carotenoids already reported to be present in CPO. Palm oil also contains significant quantities of α-carotene which also has pro-vitamin A activity (Ng & Tan, 1988). However, we have concentrated our attention on β-carotene because it is the major carotenoid present with maximum pro-vitamin A activity (Freed, 1966).

Retention of β-carotene in different cooked foods was estimated after preparation and uniform homogenization of the food item and extraction of carotenoids from an aliquot for injection and separation on HPLC.

Table 2 gives the percentage retention of total and β-carotenes in different food items subjected to different types of heat treatment. It was observed that total carotene retention ranged from 69 to 86% and β-carotene retention from 70 to 80%.

Surprisingly, cake baked at 220°C for 45 min retained 88% of the β-carotene originally present when compared with other food items which are cooked at 180°C. Since CPO is thoroughly blended with other ingredients like flour, sugar and egg, thereby avoiding direct exposure to heat, probably the retention after cooking is higher. In preparations like 'upma', 'suju

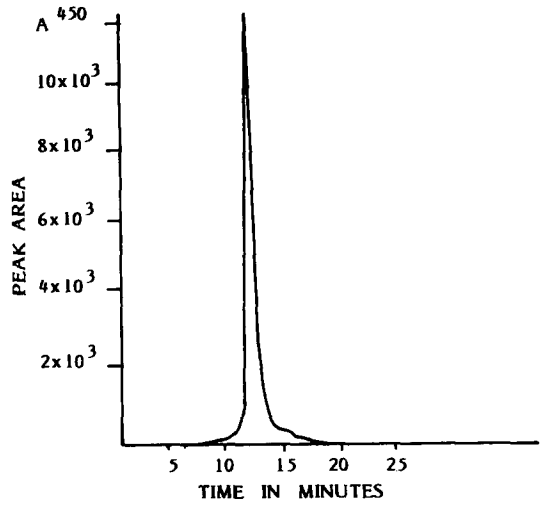

Fig. 2a. HPLC elution of standard β-carotene at 450 nm detection in 14·5 min with a mobile phase of acetonitrile/methanol/dichloromethane (60%/35%/5%) at a flow rate of 1·5 ml/min.

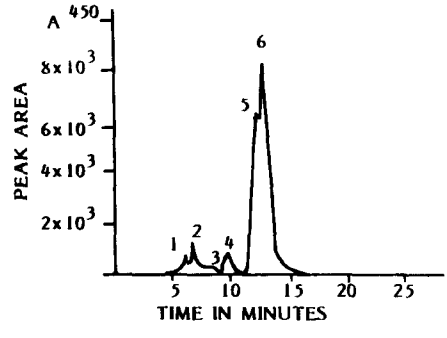

Fig. 2b. HPLC elution of CPO at 450 nm detection in 14·5 min with mobile phase of acetonitrile/methanol/dichloromethane (60%/35%/5%) at 1·5 ml/min. Peaks 1–5 are unidentified and peak 6 is β-carotene.

halwa' and 'muruku', the oil is directly exposed to the heated pan during preparation. Greater retention of total and β-carotene in the deep fried preparation 'muruku' may be due to the very short time of exposure of the oil to a temperature of 180 ± 3°C. It takes only 5 min for the oil to reach the temperature and another 2–3 min for the 'muruku' to fry. Comparatively, the amount of time taken for 'upma' and 'suji halwa' to be cooked is longer (15–20 min) and also a smaller amount of oil is being exposed to a larger surface area of heat. However, these differences are minor and it is encouraging to note that in all preparations, the retention of β-carotene is above 70%. As the content in CPO is quite substantial, one serving of each food preparation supplies more than the daily requirement of β-carotene for

TABLE 2
Percentage Retention of Total and β-Carotenes in Different Cooked Food Items and Quantity Supplied Per 100 g Serving

Recipe	*Total carotenes (% retention)*	*β-carotene (% retention)*	*Quantity supplied per 100 g serving (μg)*	*Cost per RDA serving*[a] *Rupees*[b]
Upma	69	70	1 847	0·25
Cake	86	88	6 536	0·75
Suji halwa	70	71	6 034	0·50
Muruku	76	77	4 396	0·50

[a] RDA per day per school-going child = 2 400 μg; RDA per day per pre-school child = 1 200 μg.
[b] Ten rupees = UK£0.29 = US$0.52.

all age groups (Table 2), except for 'upma' which supplied the daily requirement for a pre-school child, but not for a school-going child.

In the second part of the experiment, an attempt was made to study the loss of β-carotene after each consecutive frying. CPO was fried repeatedly five times and the content of β-carotene in different aliquots of the oil was estimated. Results are presented in Figs 3 and 4. Both for total and β-carotenes, the initial decrease after the first frying was not much when compared with the sharp fall after the second and further consecutive

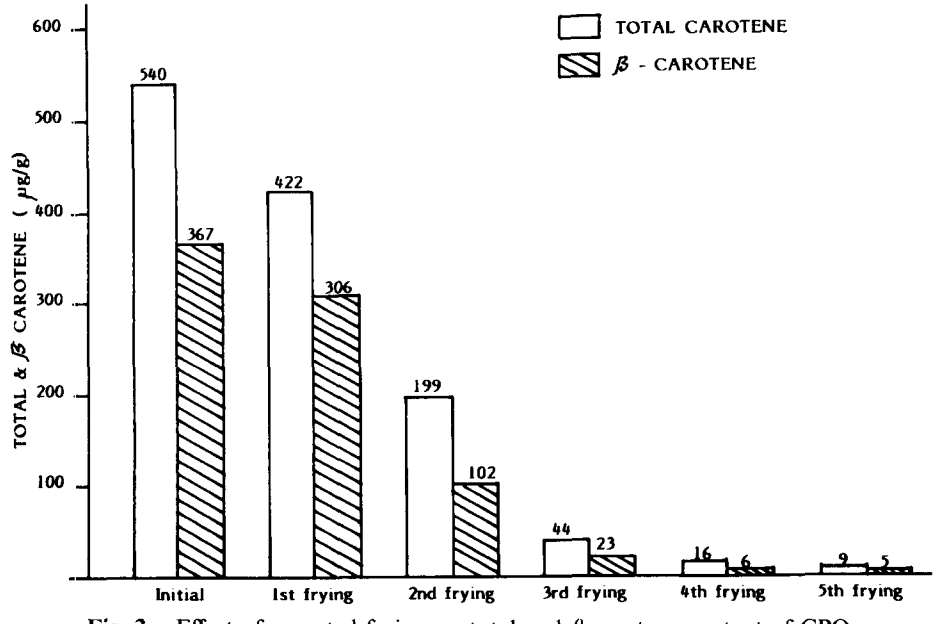

Fig. 3. Effect of repeated frying on total and β-carotene content of CPO.

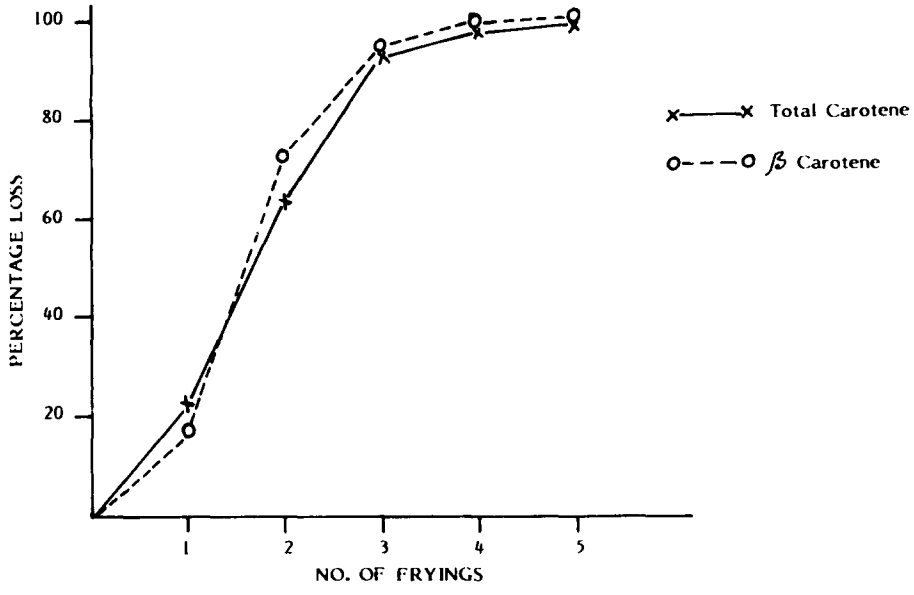

Fig. 4. Percentage loss of (×) total and (○) β-carotene due to repeated frying of CPO.

fryings. The loss of β-carotene during deep frying can be attributed to two factors, namely, loss due to heat deterioration and loss due to being incorporated into the food material being fried. At the end of the first frying, the decrease of carotenes can be attributed more to incorporation into the food material than to heat deterioration, but in further consecutive fryings, especially from the third frying stage, the loss could be attributed to heat deterioration.

Repeated heating of CPO results in oxidation of its components and fragmentation to various compounds which alter the organoleptic, chemical and physical properties of the oil (Okiy & Oke, 1986). Wong (1977) identified some stable yellow pigments of heated palm oil that are difficult to bleach and reported them to be co-oxidation products of carotene and linoleate residues. Since the concentration of carotenes is high in CPO, it was assumed that the concentrations of the breakdown products of carotenes will be more than those of linoleic acid in the oxidized samples of CPO (Wong, 1977).

These results and observations suggest that CPO is not a suitable deep frying medium, especially for repeated frying. It may be used for single frying of foods and also in food items which absorb the total amount of CPO added, e.g. 'upma', 'cake' and 'suji halwa' and which involve a short cooking time. Cooked food items had a pleasant yellow–orange colour like the added colour of turmeric or saffron. Therefore, an acceptable item like the sweets suggested above, can serve as ideal vehicles for supplementation of

β-carotene to children in deficient populations. It can, therefore, be suggested that 'suji halwa' could be an ideal choice for supplying to children in supplementary feeding programmes because it was found to be well accepted, works out to be cheap per RDA serving and it retains around 70% of its β-carotene after cooking.

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