J Amer Oil Chem Soc (2007) 84:587–596
DOI 10.1007/s11746-007-1078-9

ORIGINAL PAPER

Process Technology for the Production of Micronutrient Rich Red Palm Olein

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Received: 21 August 2006 / Revised: 29 March 2007 / Accepted: 24 April 2007 / Published online: 26 May 2007

Abstract Crude palm oil (CPO) is the richest natural source of carotenes that are destroyed in the conventional processing. There is a growing demand for nutritional products containing bioactive constituents externally fortified or preserved through modified process. A commercially viable process for the production of red palm olein (RPOn) rich in carotenes, tocols and sterols has been developed at pilot scale. The process developed involved neutralization of CPO followed by crystallization at controlled rate of cooling and deodorization of the resultant neutralized and winterized palm olein (WPOn) under controlled conditions of temperature and high vacuum. Analytical data related to micronutrients at each process step was monitored. The RPOn thus produced had not more than 0.25% of free fatty acids (FFA) and it retained more than 80% of the carotenes, about 85% of tocols and 65% of sterols originally present in the CPO. The physico-chemical characteristics of RPOn revealed that it is nutritionally of superior quality compared to that of the commercial refined bleached deodorized (RBD) palm olein currently available in the market. The carotenes, tocols and sterols profile of RPOn by HPLC showed that they were retained in their natural forms.

Keywords Carotenes · Deodorization · Differential scanning calorimetry · High-performance liquid chromatography · Red palm olein · Vitamin E

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Introduction

β-carotene and tocopherols are well known for their classical functions as provitamin A and vitamin E, respectively. They are also recognized now as potent antioxidants. CPO is the richest natural source of α-β-carotenes (500–1,500 ppm) and rich source of tocols (700–1,000 ppm) [1]. In addition to this, CPO also contains significant amounts of ubiquinones, squalene and sterols that are known for a variety of biological activities such as antioxidant, hypocholesteremic, etc. [2]. CPO with its high levels of γ-tocotrienols (500–700 ppm) is only next to rice bran oil (1,000–1,500 ppm) in this respect and no other edible oils of commercial significance contain γ-tocotrienols of this order. CPO therefore has unique combination of natural antioxidants, based on the current scientific evidences, which are known to modulate cardiovascular diseases, cancer, diabetes, cataract, aging, etc. [3, 4]. CPO with its rich β-carotenes is important in the context of vitamin A deficiency afflicting millions of children in the developing countries besides its other health promoting properties. Recently there have been attempts towards biofortification of β-carotene in mustard oil and rice through recombinant DNA technology, which is not only expensive but also involves unresolved ethical and environmental issues [5]. It is in this context, CPO particularly with its high contents of β-carotenes and tocols that are naturally present in this oil of huge commercial significance assumes importance. Currently about 30 million metric tons (MMT) CPO is produced per annum, the β-carotene content of which is sufficient to meet the vitamin A requirement of the world population. However, the conventional process of refining presently practiced in the industry removes almost the entire β-carotenes and substantial amount of other micronutrients [1–3, 6–11]. Alternative process to produce

carotene rich RPOn has been attempted in Malaysia [3]. The ''Caroteno'' brand developed by PORIM (Palm Oil Research Institute of Malaysia) has employed molecular distillation technology and the product has become very expensive for common use [11]. Conventional refining of CPO makes the oil bland, colorless with about 60–80% reduction in carotene content and nutritionally poor with about 10–35% reduction in the content of micronutrients such as tocols and sterols [1–3, 8, 10], as a result of using extreme process conditions above 200 °C to meet with commercial specifications prevailing now for the RBD palm olein. Novel product concepts are now emerging [11–14] based on minimal processing thereby retaining naturally present bioactive micronutrients to satisfy health conscious consumers. This paper describes a new process at pilot scale, which enables to produce RPOn that retains most of its natural carotenes, tocols and sterols at commercially competitive price.

Materials and Methods

Raw Material

CPO with less than 3% FFA was procured from M/s Godrej Agrovet Pothepally, Andhra Pradesh (India).

Chemicals

Tocopherol and tocotrienol standards were obtained from Merck, Darmstadt, Germany. Fatty acid methyl ester (FAME), carotene and sterol standards were procured from Sigma chemicals, (St Louis, MO). All other chemicals used were of laboratory grade.

Process Description at Pilot Scale

All the equipments used to develop this process were of standard make and were of pilot scale models of commercial systems with process controls. On an average, ten pilot scale trials were conducted for each unit operation.

Neutralization

The equipment used for neutralization was FT66, Neutralizer/Washer/Bleacher (Armfield, Ringwood, Hampshire, UK). The main parts of this equipment are reactor vessel, agitator drive, bleaching earth hopper, reagent tank, pressure leaf filter, liquid ring vacuum pump, filter pump and control console. The reactor vessel is designed to process 25 L per batch. The internal parts of this reactor vessel include; agitator paddle, baffle arrangement, electrical heating element and cooling coil. Twenty kilograms of the CPO was charged into the neutralizer by applying vacuum (70 mmHg). The CPO was heated under vacuum with gentle agitation to 80 °C. Calculated amount of NaOH (115 g) was dissolved in required quantity (1 L) of distilled water and heated to 80 °C in the reagent vessel and slowly admitted to the reactor vessel and mixed well under slow stirring maintaining vacuum. After 10 min, the stirring was stopped and the oil was cooled to 70 °C using chilled water and vacuum released at 70 °C and further cooled to 48–50 °C. After settling for a period of 2 h, the soap stock was removed from the bottom of the vessel. The separation of the soap stock was completed before the temperature dropped below 48 °C. After soap separation, the oil was washed free of alkali and soap using hot water at 80 °C under vacuum. Four to five washings were required with oil to water ratio of 7:1 for each washing.

Crystallization

Crystallizer of 50 kg capacity (M/s Thermosystems, Trivandrum, India) was used for crystallization. This equipment is a vertical, cylindrical, jacketed stainless steel vessel with scraped surface blade type of agitator, with a variable speed drive. The agitator is designed to scrape the inner surface of the crystallizer so as to avoid crystal accumulation and consequent heat transfer problems. The entire heating and cooling system is controlled by PID (Proportional Integral Derivative) controller having facility to preprogram the heating and cooling rates as per the requirement. Thermocouple probes are provided at two positions (top, and bottom) through the agitator shaft and connected to the PID controller so as to maintain the temperature as per the heating/cooling programme uniformly throughout the mass. Neutralized palm oil (NPO) was fed into the crystallizer and heated to 70 °C by admitting steam into the jacket, over a period of 60 min and held at 70 °C for 15 min to destroy any crystal. Oil was then cooled to 25 °C at different cooling rates of 15, 17, 20, 25, 30 and 35 °C h^{-1} by admitting chilled water into the jacket. Different rate of cooling was employed here, to arrive at the optimum ΔT at which yield of β crystals was maximum. The heating and cooling, at the set rate was controlled by PID through solenoid valve. When the temperature of the oil reached 25 °C the crystallized mass was taken out for separation.

Filtration

The crystallized palm oil was then separated into palm stearin (PS) and olein using rotary drum vacuum filter (M/s Thermosystems, Trivandrum, India), which has a rotating

drum on which the filter medium, in this case ordinary 100% polyester cloth, is used. The drum is rotated by means of a variable speed drive. Rotating drum, partially submerged in the oil, uses vacuum to draw the oil through the filter of the drum. The olein fraction was separated and the filtrate discharged to a holding tank. The PS cake on the rotating drum was scraped off by knife blade continuously from the outer surface of the drum and collected in the receiving trough. The liquid palm olein was then subjected to deodorization.

Deodorization

Deodorization was carried out in a FT 68 Deodorizer (Armfield, Ringwood, Hampshire, UK). It is a cylindrical stainless steel vessel of 30 L capacity, which contains a specially designed baffle system to sparge steam that promotes efficient mixing of the oil and live steam. The vessel is provided with electrical heating element and a stainless steel internal cooling coil. The high vacuum required for the process is achieved by combination of a multi-stage liquid ring vacuum pump in series, and an efficient steam ejector. Twenty-five kilograms of WPOn was taken for deodorization in each batch. The deodorization vessel was evacuated first to 1 mmHg by operating the water ring vacuum pump and steam ejector. After reaching the vacuum to 1 mmHg, the oil was charged into the deodorization vessel using vacuum. The deodorization was conducted at 130, 140 and 150 °C for 2 h. Different deodorization temperatures were used here to arrive at the minimal temperature at which retention of carotene was maximum without any unpleasant odor. The oil was heated to 70 °C using electric heating followed by live steam sparging at 0.35 kg h^{-1}. Further heating to set temperature was achieved by electric power and live steam sparging. Samples were withdrawn every half an hour through the sampling valves to monitor the progress of deodorization for each experiment.

Analyses

FFA%, iodine value (IV), moisture%, melting point (MP), Fe and Cu contents were determined by following AOCS method [15].

Fatty Acid Composition

FAME were prepared by esterifying with alcoholic sulfuric acid reagent according to the IUPAC procedure [16]. A Hewlett Packard 5890 series 11 GC (Avondale, PA) equipped with an FID was used for GC analysis of the methyl esters. Methyl esters were analyzed on a Hewlett Packard Free Fatty Acid Phase (cross-linked FFAP) column (30 m × 0.53 mm × 1.0 μm). The injection and detector temperatures were maintained at 250 and 300 °C, respectively. The flow rate of the carrier gas (nitrogen) was 20 mL min^{-1}. The oven temperature was programmed from 100 to 180 °C at the rate of 5 °C min^{-1}. FAME were identified by using authentic standards, and the peaks were quantified by using digital integration. Fatty acid (FA) levels were reported as relative proportions of the total composition.

Carotenes, Tocols and Sterols Analyses

The analysis was performed with a Shimadzu make HPLC binary system (Kyoto, Japan) with LC-10 AD model pump, a 7125 model Rheodyne injector (Cotati, CA, USA) fitted with a 20 μL sample loop, a SPD-10 A UV-visible detector, with a C-R7Ae plus integrator for data acquisition and display. Analysis for carotenes and sterols were carried out using a reverse-phase zorbax ODS column (4.6 mm i.d. × 25 cm) with a guard column of CLC-ODD (4 mm i.d. × 1 cm). Carotene analysis was carried out according to the procedure of Ben-Amotz and Fishler [17]. The mobile phase used for carotenes was acetonitrile, dichloromethane, methanol (70:20:10 by vol) with a flow rate of 1 mL min^{-1}. The UV-VIS detector was set at 450 nm with a detector sensitivity of 0.005 AUFS. The solvent system used for sterols was methanol, water (96.5:3.5 by vol) with a flow rate of 1.2 mL min^{-1}. The UV detector was set at 206 nm [18]. For the analysis of tocols a Shim-pack [LC-NH2 (M)] column (4.6 mm i.d. × 25 cm) was used in the normal phase with the solvent system, *n*-hexane, isopropanol (96:4 by vol) and a flow rate of 1 mL min^{-1}. The UV detector was set at 297 nm [4]. Quantification of all the nutrients was carried out from standard curves using authentic standards.

X-ray Diffraction Analysis

The polymorphs of PS crystals were determined using a Philips 1710 X-ray diffraction (Almelo, The Netherlands) emitting Cu–Kα radiation (λ = 1.5418 Å). Data were collected from 5 to 45 $2\theta°$ with a step width of 0.04° and step time of 1 s operating at room temperature. X-ray data were processed by a computer, programmed to calculate absorption intensity-back ground, intensity and peak width in degrees for each crystalline form and the relative content of α, β and β' crystals. The β was calculated from the intensity of the short spacing at 4.6 Å. The β' fraction was calculated from the intensities of the short spacing of 3.8 and 4.2 Å. The α fraction was calculated from the intensities of the short spacing of 4.15 Å [19].

Differential Scanning Calorimetry

Melting characteristics of CPO, NPO, PS, WPOn and RPOn were studied using a DSC (Mettler Toledo DSC 821, Schwerzenbach, Switzerland). Samples were subjected to the following temperature program: holding at 80 °C for 30 min, cooling from 80 to –30 °C at a rate of 10 °C min^{-1}, and holding at –30 °C for 2 min. The same sample was then heated from –30 to 80 °C at the rate of 5 °C min^{-1} [20]. Heating and cooling thermogram traces were recorded.

Statistical Analysis

The analyses were conducted independently for five trials and the results were analyzed statistically using software option Origin version 6.0, Microcal (Northampton, MA, USA).

Results and Discussion

Neutralization

The effect of neutralization on the reduction of FFA% showed that a tenfold reduction in the percentage of FFA had occurred for NPO when compared to that of CPO (Table 1). The soap-settling step after neutralization was monitored at 48–50 °C as it was observed that, below this temperature regime, PS was found to crystallize thereby increasing the neutral oil loss. The average refining loss at the neutralization step was found to be 1.5 kg and therefore, based on the FFA% in CPO (3%), the average refining factor arrived at was 2.5, which is within the accepted limit of refining loss in the edible oil industry [2, 11].

Crystallization

Three types of fractionation namely dry fractionation, detergent fractionation and solvent fractionation are employed for separation of palm olein and PS [2]. Detergent fractionation requires several unit steps and treatment with chemicals and removal of these chemicals with hot water washing and drying of oil, which results in the significant reduction of carotenes, tocopherols and tocotrienols [1–3, 6–9]. Crystallization using solvent fractionation requires evaporation/distillation to remove the solvent, which also results in the reduction of carotenes and tocols. For the present process, dry fractionation was found to be suitable in order to retain maximum carotenes and tocols. In this process, crystallization was conducted at different cooling rates from 15 to 35 °C h^{-1}. Palm oil is composed of mixed triglycerides and thus can crystallize in several polymorphic forms designated as α, $\beta'$and β in the order of increasing stability and melting points. To have good separation, it is desirable to have maximum β' polymorphic form because β' crystals agglomerate into large, firm porous clusters [21] that facilitate subsequent filtration. Cooling rate is a critical parameter that influences nucleation and crystal growth of the glycerides. Slow cooling with proper mixing is essential for the formation of desired polymorps. In this process, at the cooling rate of 17 °C h^{-1}, maximum palm olein yield (70%) and PS with maximum β' polymorphic form were obtained. As the cooling rate increased from 20–35 °C h^{-1} the olein yield decreased to 68–64%. Results indicated that β' polymorph was dominant in all the PS samples collected at different cooling rates. It was further observed that with increase in the rate of cooling, more of the unstable forms were noticed in PS (Fig. 1).

Filtration

Filtration was carried out using the rotary drum vacuum filter as described before. Porosity and stability of crystal is important to obtain dry PS with maximum olein yield, on filtration. β' is the most suitable polymorphic form to meet such process requirements. As crystallization is a function of rate of cooling, ideally, the entire filtration operation is

Table 1 Changes in the physicochemical characteristics of CPO and its fractions during processing

Characteristics	CPO	NPO	WPOn	NPS	RPOn
FFA (%)	3.00 ± 0.18	0.30 ± 0.02	0.50 ± 0.02	0.30 ± 0.01	0.25 ± 0.02
Moisture (%)	0.25 ± 0.02	0.26 ± 0.02	0.06 ± 0.01	0.28 ± 0.01	0.02 ± 0.01
IV	50.00 ± 0.34	50.00 ± 0.18	57.00 ± 0.18	44.00 ± 0.18	57.00 ± 0.07
MP (°C)	37.00 ± 0.29	37.00 ± 0.29	15.00 ± 0.23	52.00 ± 0.23	15.00 ± 0.23
Cloud point (°C)	_	_	_	_	8.00 ± 0.12
Fe (ppm)	5.00 ± 0.41	1.30 ± 0.18	0.80 ± 0.09	2.60 ± 0.12	0.80 ± 0.07
Cu (ppm)	0.47 ± 0.02	0.15 ± 0.01	0.06 ± 0.01	0.17 ± 0.01	0.06 ± 0.01

The values are expressed as mean ± SD (n = 5)

CPO crude palm oil, *NPO* neutralized palm oil, *WPOn* winterized palm olein, *NPS* neutralized palm stearin, *RPOn* deodorized red palm olein, *IV* iodine value, *MP* melting point

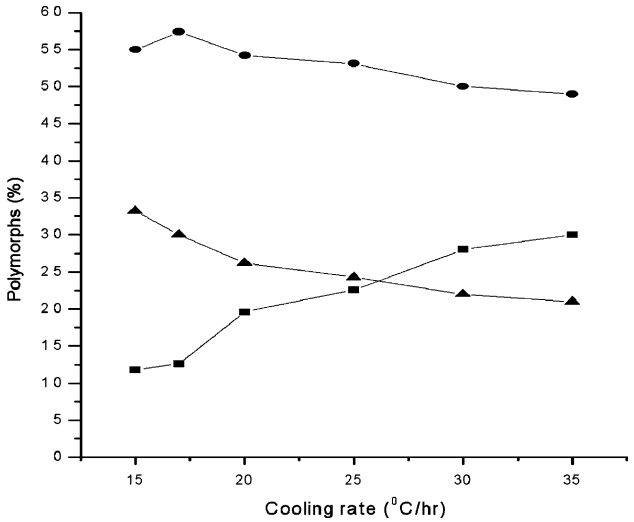

Fig. 1 Polymorphic forms of palm stearin collected at different cooling rates. *Filled square* α, *filled circle* β′, *filled triangle* β

required to be completed at the specified temperature of 25 °C at which maximum crystals of β′ occurred. Otherwise, polymorphic change could occur leading to transformation of β′ to other undesirable crystals. Consequently, the yield of palm olein could be affected and resulting in soft PS. In the present process, it was observed that no significant change occurred for 2 h during which the filtration was completed. The filtration rate as well as the separation efficiency was found to be dependent on the crystal morphology. The larger the crystal size (β form) lesser the intracrystalline packing, and therefore the separation was slower and incomplete leading to lower yield of olein and soft PS. This was due to olein entrained in the crystal mass. The PS cake with more of β′ acted as the filter medium that facilitated filtration. The optimum thickness of PS cake was found to be between 6–8 mm so as to reduce resistance to filtration under the conditions of pilot scale trials reported here. The PS thus obtained was enriched in desirable β′ polymorphs with least amount of occluded olein. The yield of PS and olein under these conditions was 30 and 70%, respectively, which is comparable with that of the yield achieved in palm oil industry using vacuum drum filtration [2, 3]. Totally automated membrane filtration technology is being preferred now by the industry to increase the yield of olein to 75% or more to obtain very hard PS [2, 22].

Deodorization

Deodorization is employed primarily to remove trace volatile components that contribute to off flavor. These compounds are ketones, aldehydes, alcohols, FFAs, etc. A significant reduction in color is also brought about at this stage through thermal bleaching of pigments like carotenoids and chlorophylls under the standard conditions of deodorization practiced in the edible oil industry at temperature more than 180 °C and vacuum less than 5 mmHg [6]. Under such extreme conditions of deodorization, nutritionally important micronutrients such as carotenes, tocols, and sterols are either destroyed or stripped off from the oil resulting in nutritionally poor end product [1–3, 6–10]. Almost entire quantity of carotenes and 20–30% of tocols and sterols are normally removed under the deodorization conditions currently practiced in edible oil industry [23]. In the present process, conditions of deodorization at low temperature and at high vacuum were optimized to preserve the naturally present carotenes, tocols and sterols in the palm olein. Considering the sensitivity of these micronutrients to temperature, particularly of carotenes, protocols for raising the preheating temperature to the desired level and maintaining the temperature of deodorization coupled with vacuum requirements was optimized in this process to preserve the micronutrients in the product. The WPOn was charged into the deodorizer under vacuum and subsequently vacuum was maintained at 0–1 mmHg with slow heating to 70 °C until the residual moisture was eliminated. Further heating to the desired temperature was done strictly under the specified vacuum with admission of live steam at the rate of 0.35 kg h^{-1}. Under this heating regime, the deodorization was conducted at 130, 140 and 150 °C for 2 h. Samples were drawn through the sampling port at every 30 min interval to monitor the progress of deodorization and micronutrient levels.

Physical and Chemical Characteristics of Palm Oil and its Fractions During Processing

Major parameters that govern physico-chemical properties of edible oil such as FFAs, IV, MP, cloud point and heavy metals, as influenced by processing are presented in Table 1. It could be seen that the major changes on these parameters happened during crystallization and subsequent separation into PS and palm olein. IV was increased to 57 in the olein fraction with a proportionate decrease of that in the PS. The MP on the contrary was significantly higher for PS (52 °C) and lower for olein (15 °C). The difference in MP and IV as presented for olein and PS fractions was reflected in the cloud point of the olein fraction, which was lowered to 8 °C. The heavy metals like Fe and Cu that influences the stability of oil were tend to concentrate in the PS fraction. Physical and chemical characteristics of PS and olein fractions presented in the table clearly established the process of crystallization was well optimized to produce a hard PS fraction and liquid olein as practiced in the palm oil industry [22].

FA composition of CPO and its fractions are given in Table 2. As in the case of physico-chemical characteristics, there was a significant partitioning of glycerides having unsaturated FAs and those having more of saturated ones. The physical process of crystallization and separation into palm olein and PS could bring about the segregation of these glycerides that was reflected in the FA composition of the liquid olein and solid PS. It could be seen from the results that the major saturated FA, namely palmitic acid, was concentrated in the PS fraction with 56.7% as against 40.1% in the olein whereas its original concentration in the CPO was 45%. Conversely the most abundant unsaturated FA $C_{18:1}$ was enriched in the olein fraction with 44.8% against 29.8% in the PS fraction. A similar trend was also observed in the case of linoleic acid ($C_{18:2}$). The FA composition of the fractions as presented here is comparable with the reported values [24]. It is also obvious from the results that the process steps such as neutralization and deodorization did not alter the FA composition in any significant manner.

The melting thermograms obtained using DSC of CPO, PS, WPOn and RPOn are presented in Fig. 2. The different polymorphic forms α, β' and β have hexagonal (H), orthorhombic perpendicular ($O_{\perp}$), and triclinic parallel ($T_{\parallel}$) subcell structures, respectively. In the hexagonal subcell structure, the 2D lattice is hexagonal and chain packing is loose and specific chain–chain interactions are lost. The 2D lattice of an orthorhombic perpendicular ($O_{\perp}$), subcell structure is rectangular and this represents a tightly packed lattice with specific chain–chain interactions. Triclinic parallel subcell structure ($T_{\parallel}$) has an oblique 2D lattice and represents tightly packed chains, in which there are specific chain–chain interactions [21]. Melting curve of CPO showed two major endotherm regions corresponding to endothermic transitions of the olein (lower-temperature peak) and PS (higher-temperature peak) fractions which represent polymorphs β'_1 and β_1 while the low temperature peaks represent polymorphs β'_2 and α. PS showed both endotherm regions, the higher region was distinguished by a tall peak and two small fusion peaks preceding the low temperature region, which is characteristics of PS as reported by others [25]. However WPOn and RPOn showed similar melting curves which showed only one major endotherm with two fusion peaks in the lower temperature region (olein fraction) which represent polymorphs β'_2 and α. The results showed that there was no significant change in the heating thermograms of WPOn and RPOn indicating the absence of new compounds generated by the oxidation of carotenes and unsaturated FAs during deodorization [26].

Micronutrient Levels in RPOn as Influenced by Processing

The major objective of this work was to develop a commercially feasible process to produce micronutrients enriched palm olein. The process conditions therefore were modified as discussed elsewhere to retain maximum amount of the major micronutrients such as carotenes, tocols and sterols. Carotene levels at each process step are presented in Table 3. Individual carotenes were separated and quantified by HPLC. The results obtained demonstrate that CPO used for the present study contained 562 ppm carotenes. It could be seen that the major carotenes were α (272 ppm) and β (282 ppm) with lycopene (8 ppm) as the minor component. The carotenes of provitamin A value therefore accounted for about 98% of the total carotenes. The first process step involved was neutralization of CPO using alkali as described before. Unlike in the normal industrial chemical refining, the neutralization in this process was carried out under vacuum of 70 mmHg at 80 °C. Under the conditions optimized, there was only 2% loss of carotenes at this stage. In the crystallization process there was an enrichment of carotenes in the olein fraction with approximately 15% increase from its level in CPO. This could be attributed to the partitioning of carotenes in favor of the liquid fraction due to their higher solubility [27]. Deodorization is the harshest operation in edible oil refining in terms of exposure of the product to temperature and time. Usually deodorization is conducted at 180–200 °C for more than 3 h in chemical refining using live steam. Most of the nutritionally important micronutrients are lost at this stage [1–3, 6]. Aliyas et al. [7] during their investigations on changes of β-carotene content on heating of

Table 2 Fatty acid profiles of CPO and its fractions

Fatty acid	CPO (wt %)	NPO (wt %)	WPOn (wt %)	NPS (wt %)	RPOn (wt %)
12:0	0.5 ± 0.01	0.5 ± 0.03	0.1 ± 0.02	1.2 ± 0.12	0.1 ± 0.02
14:0	1.2 ± 0.12	1.1 ± 0.07	1.1 ± 0.06	1.4 ± 0.13	0.8 ± 0.13
16:0	45.0 ± 0.23	45.6 ± 0.10	40.6 ± 0.07	56.7 ± 0.27	40.1 ± 0.19
18:0	3.2 ± 0.06	3.2 ± 0.07	3.2 ± 0.03	4.0 ± 0.24	3.1 ± 0.26
18:1	40.2 ± 0.09	39.8 ± 0.06	44.5 ± 0.35	29.8 ± 0.13	44.8 ± 0.13
18:2	9.6 ± 0.05	9.6 ± 0.04	10.2 ± 0.24	6.8 ± 0.13	10.8 ± 0.15
Others	0.3 ± 0.03	0.2 ± 0.05	0.3 ± 0.02	0.1 ± 0.03	0.3 ± 0.03

The values are expressed as mean ± SD (n = 5); for other abbreviations see Table 1

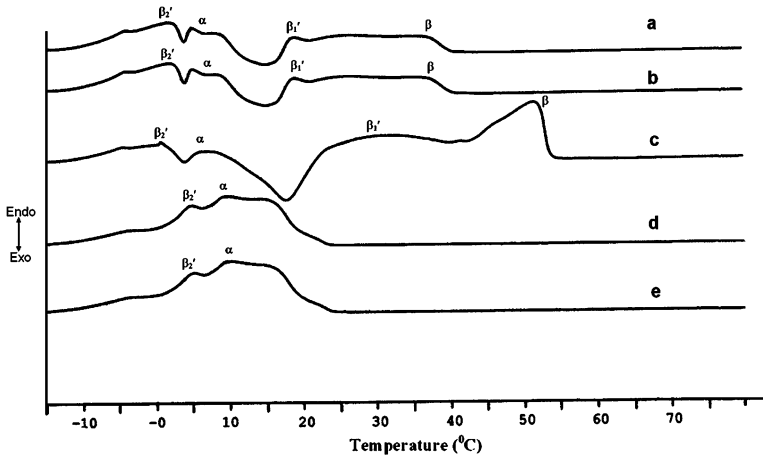

Fig. 2 The DSC melting thermograms of *a* crude palm oil, *b* neutralized palm oil, *c* neutralized palm stearin, *d* neutralized winterized palm olein, *e* deodorized red palm olein

Table 3 Changes in carotenes in CPO and its fractions by HPLC

Sample	α-Carotene (ppm)	β-Carotene (ppm)	Lycopene (ppm)	Total (ppm)
CPO	272 ± 3.30	282 ± 3.50	8 ± 1.45	562
NPO	269 ± 2.70	278 ± 2.54	4 ± 0.35	551
NPOn	301 ± 3.70	312 ± 3.67	18 ± 0.35	631
RPOn	212 ± 2.50	233 ± 2.54	5 ± 0.90	450
Commercial sample	ND	ND	ND	

The values are expressed as mean ± SD (n = 5)

ND not detectable

red palm olein observed 59% reduction on heating at 200 °C. However, they also observed, increasing the heating time from 30 to 120 min resulted in 3 or 6% reduction of β-carotene at 50 or 100 °C. Okiy and Oke [9] noticed destruction of carotenes during heating of crude palm oil at different temperatures for 2 h and the extent of reduction was found to be 23.38, 83.66 and 92.11% at 100, 150 and 200 °C, respectively. The deodorization step in crude palm oil refining resulted in the production of a light colored, bland RBD palm oil and it was observed that palm oil refining removes 63 and 86% of carotenes at 180 and 200 °C, respectively [2, 6]. In the present process, the focus was to preserve the micronutrient particularly carotenes in the end product. Among the micronutrients present in edible oils, carotenes are the most sensitive to heat, oxygen and light with duration of exposure being critical. The conditions therefore were standardized in terms of minimum temperature, time and maximum vacuum to reduce the impact of deodorization on carotenes. Based on the several pilot scale trials as described before, it was combination of temperature, vacuum and duration that could maximize the retention of carotenes; at the same time the product was free of unpleasant odor, which is the main objective of the deodorization step. Under the conditions optimized, the results presented in the table indicate that about 80% of the carotenes were preserved after deodorization at 140 °C under 1 mmHg vacuum for duration of 2 h (Table 3). Time course retention of carotenes during deodorization is presented in Fig. 3. It was observed that at

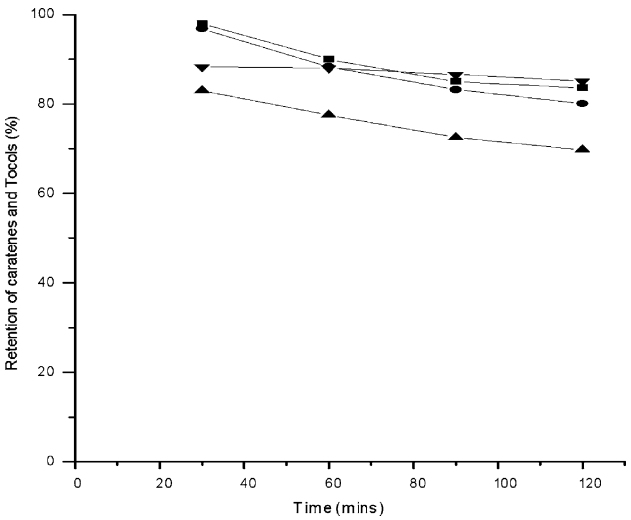

Fig. 3 Retention of carotenes and tocols in red palm olein during deoderization. *Filled square* carotenes at 130 °C, *filled circle* carotenes at 140 °C, *filled triangle* carotenes at 150 °C, *filled inverted triangle* tocols at 140 °C

130 °C though the retention was higher (85%) there was characteristic fruity odor even after 2 h of deodorization. At 150 °C there was a faster rate of destruction of carotenes with final value of 70% retention at the end of 2 h. It could be seen from Fig. 3 that the rate of destruction was faster during the first 1 h and subsequent rate of reduction was far lower than that of the first hour. It was further

noticed that there was no preferential rate of destruction for α and β carotenes under the process conditions employed here. The commercially produced palm olein in the market has also been analyzed for the carotene and found that carotenes were not at the detectable levels and therefore the product is pale yellow in color as against the deep orange color for the deodorized RPOn produced through the process described here. Tocols content of the samples from each process step were analyzed by HPLC and the results are shown in Table 4. The palm oil is one of the few edible oils that contain tocotrienols in substantial quantity in addition to commonly occurring tocopherols. The analytical protocols standardized here could resolve tocols into α-T, α-T_3, βT_3 + γT, γT_3 and δT_3 in the increasing order of retention time. It is evident from the result that CPO is also one of the rich source of tocols particularly tocotrienols. The total tocols content in CPO used for the process here was 869 ppm of which γ-T_3 accounted for about 60% followed by α-T_3 (15%), δ-T_3 (12%) and α-T (12%). Total tocotrienols thus accounted for 85% of the total tocols. The nutritional significance of tocotrienols is that they are most potent biological antioxidants that modulates cardio vascular disease, immunity, etc. [4]. Therefore, their retention in the product was one of the objectives of this work. The tocols followed a similar trend as that of carotenes under the different process steps employed here. However, the final product namely deodorized RPOn had 85% of the total tocols present in the CPO. As in the case of carotenes, the individual tocols also did not show any preferential rate of retention. In contrast to the product developed through the process reported here, the commercially produced palm olein (market sample) contained 291 ppm of tocols indicating that the process developed here is far more superior in terms of tocols content (739 ppm). The rate of retention during the deodorization period of 2 h for tocols is presented in Fig. 3, which is comparable with that of carotenes, though there was higher percentage of retention. Earlier research by Barrera et al. [8] demonstrated that tocopherols were lost rapidly for palm olein at 180 °C heating for varying time durations. For 2 h, reduction was 32.18%, for 4 h 55.79%, for 6 h 84.97%, for 8 h trace levels and for 10 h, non-detectable levels. Choo et al. [12] reported 35.07% reduction in tocols in RBD palm olein from palm oil refinery in connection with their investigations on production and applications of deacidified and deodorized red palm oil. Studies by Ferrari et al. [23] on minor constituents of vegetable oils during industrial processing revealed reduction in the levels of tocopherols and tocotrienols by about twofold after complete refining of corn oil and by about 1.5-fold in soybean and rapeseed oils. Verhe et al. [28] noticed significant loss in the tocopherol mass balance in the range of 25–35% with several transformation reactions such as oxidation of tocopherols at high temperature (180–240 °C) and formation of steradienes duing the refining of vegetable oils such as palm, sunflower and flax seed oils.

The phytosterols have recently received nutritional significance due to their hypocholesteremic property [29] and therefore sterols content were also monitored in this process. The results are presented in Table 5. The total sterols was separated and quantified by HPLC and it was found that CPO contained stigmasterol, campesterol and β-sitosterol in the increasing order of abundance with total of 646 ppm. As in the case of other micronutrients reported here, there was a reduction of the sterols at various process steps with maximum decrease at the deodorization stage. Only about 65% of the original sterols were retained in the deodorized RPOn, the retention being lower than those for carotenes and tocols. The commercial sample analyzed was found to contain far less than that of the RPOn reported here. However palm oil is not a good source for sterols. Ferrari et al. [23] have reported the effects of individual steps of industrial refining on the alteration of selected minor constituents of oils, such as corn, soybean and rapeseed oils and have observed that total sterols decreased by 18–36% in the fully refined oils, compared with the crude oils. According to De Greyt and Kellens [6] deodourization of soybean oil under varying process conditions of 220–260 °C temperature, 1.5 mbar pressure and 1.5% steam resulted in a 10–35% reduction of the total sterol content.

In the recent years, biologically active micronutrients in oilseeds and edible oils and other natural products assumed significance due to their preventive and modulating prop-

Table 4 Changes in tocols in CPO and its fractions by HPLC

Sample	α-T (ppm)	α-T_3 (ppm)	β-T_3 + γT (ppm)	γ-T_3 (ppm)	δ-T_3 (ppm)	Total (ppm)
CPO	107 ± 2.50	122 ± 3.60	15 ± 0.79	512 ± 6.67	113 ± 3.60	869
NPO	105 ± 2.30	119 ± 3.50	13 ± 0.72	508 ± 7.30	110 ± 2.54	855
WPOn	120 ± 2.40	134 ± 2.90	21 ± 1.10	537 ± 9.46	120 ± 3.08	932
RPOn	76 ± 1.87	86 ± 2.12	8 ± 0.38	457 ± 7.30	112 ± 1.87	739
Commercial sample	7 ± 0.70	4 ± 0.35	ND	227 ± 2.12	52 ± 1.22	290

The values are expressed as mean ± SD (n = 5)

α-T α-tocopherol, *α-T₃* α-tocotrienol, *β-T₃* β-tocotrienol, *γ-T* γ-tocopherol, *γ-T₃* γ-tocotrienol, *δ-T₃* δ-tocotrienol, *ND* not detectable

Table 5 Sterols content in CPO and its products by HPLC

Sample	Stigmasterol (ppm)	Campesterol (ppm)	β-sitosterol (ppm)	Total (ppm)
CPO	142 ± 1.58	172 ± 3.08	332 ± 4.58	646
NPO	138 ± 0.70	170 ± 3.50	328 ± 5.19	636
WPOn	123 ± 1.87	136 ± 1.80	312 ± 4.60	571
RPOn	68 ± 0.70	118 ± 1.80	243 ± 4.90	429
Commercial sample	ND	ND	215 ± 3.50	215

The values are expressed as mean ± SD (n = 5)

ND not detectable

erties of various degenerative diseases [23]. Consequently, there have been attempts to isolate these micronutrients and fortify in the food products to enhance their nutritional values. In this context, CPO which is the richest natural source of carotenes and rich source of tocols particularly tocotrienols was used to develop a functional food rich in these nutrients. However the conventional processing employed is not designed for preserving the bioactive molecules. Considering the emerging trend on health care, end products with maximum retention of the originally present micronutrients are the current consumer requirement. In the case of palm oil, there have been attempts to develop RPOn containing most of the carotenes and tocols present in the CPO. Ooi et al. [11] reported a method using molecular distillation to produce red palm olein containing more than 500 ppm carotenes and 600 ppm tocols. Molecular distillation is an expensive process and therefore the product is prohibitively costly and thus the process is commercially unviable. In another report, modified deodorization of palm olein was described, preceded by degumming, bleaching and chemical deacidification steps [13]. A low temperature deodorization by this process was reported to retain 70–85% of carotenes and further details are not available. For the process reported here, detailed studies were conducted to optimize process conditions to retain maximum content of all the important biologically active constituents like carotenes, tocols and sterols for the first time by a commercially feasible process as described here. Relative abundance of carotenes, tocols and sterols as presented in this paper are in agreement with those of other reports [11]. Thus the deodorized RPOn produced by the process reported here is rich in α and β carotenes (445 ppm), tocols (739 ppm) with 85% tocotrienols and sterols (428 ppm). The process developed here has been released to the industry for commercial production of RPOn.

Acknowledgments This process was developed by the funding from Technology Mission on Oil Seeds (TMO), Government of India, and authors are thankful to the funding agency.

References

1. Goh SH, Choo YM, Ong SH (1985) Minor constituents of palm oil. J Am Oil Chem Soc 62:237–240
2. Basiron Y (2005) Palm oil. In: Shahidi F (ed) Bailey's industrial oil and fat products: edible oils, vol 2, 6th edn. Wiley Interscience, New Jersey, pp 374–380
3. Bonnie TYP, Choo YM (2000) Valuable minor constituents of commercial red palm olein: carotenoids, vitamin E, ubiquinones and sterols. J Oil Palm Res 12:14–24
4. Medina-Juarez LA, Gamez-Meza N, Ortega-Garcia J, Noriega-Rodriguez JA, Angulo-Guerrero O (2000) Trans fatty acid composition and tcopherol content in vegetable oils produced in Mexico. J Am Oil Chem Soc 77:721–724
5. Wittenberg L (1999) GM rice—a golden opportunity. Food Manuf 74:23
6. De Greyt W, Kellens M (2005) Deodourization. In: Shahidi F (ed) Bailey's industrial oil and fat products: processing and technologies, vol 5, 6th edn. Wiley Interscience, New Jersey, pp 352–357
7. Alyas SA, Abdulah A, Aini Idris N (2006) Changes of β-carotene content during heating of red palm olein. J Oil Palm Res Special Issue-April:99–102
8. Barrera-Arellano D, Ruiz-Mendez V, Velasco J, Marquez-Ruiz G, Dobarganes C (2002) Loss of tocopherols and formation of degradation compounds at frying temperatures in oils differing in degree of unsaturation and natural antioxidant content. J Sci Food Agric 82:1696–1702
9. Okiy DA, Oke OL (1986) Some chemical changes in heated crude palm oil. Food Chem 21:161
10. Abidi SL (2003) Tocol-derived minor constituents in selected plant seed oils. J Am Oil Chem Soc 80:327–333
11. Ooi CK, Choo YM, Yap SC, An MA (1996) Refining of red palm oil. Elaies 8:20–28
12. Choo YM, Ma AN, Yap SC, Ooi CK, Basiron Y (1993) Production and applications of deacidification and deodorized red palm oil. Palm Oil Dev 19:30–34
13. Mohd.Suria AY, Majid R, Ismail R (1996) Production of high carotene palm olein using moderate deodorization temperatures. Palm Oil Dev 23:7–9
14. Berger KG (1998) Recent results on palm oil uses in food products. Palm Oil Dev 29:7–12
15. AOCS (1998) Method Cc 3–25 official methods and recommended practices of the American Oil Chemist's Society, 5th edn. In: Firestone D (ed) American Oil Chemists' Society, Champaign
16. IUPAC (1987) In: Paquot C, Hautfenne A (eds) Standard methods for the analysis of oils and fats and derivatives, 7th edn. Blackwell, London
17. Ben-Amotz A, Fishler R (1998) Analysis of carotenoids with emphasis on 9-cis β-carotene in vegetables and fruits commonly consumed in Israel. Food Chem 62:515–520
18. Holen B (1985) Rapid separation of free sterols by reversed phase high performance liquid chromatography. J Am Oil Chem Soc 62:1344–1346
19. Zeitoun MA, Neff WE, List GR, Mounts TL (1993) Physical properties of interesterified fat blends. J Am Oil Chem Soc 70:467–471

20. Toro-Vazquez JF, Briceno-Montelongo M, Dibildox-Alvarado E, Charo-Alonso M, Reyes-Hernandez J (2000) Crystallization kinetics of palm stearin blends with sesame seed oil. J Am Oil Chem Soc 77:297–309
21. Metin S, Hartel RW (2005) Crystallization of fats and oils. In: Shahidi F (ed) Bailey's industrial oil and fat products: chemistry. properties and health effects, vol 1, 6th edn. Wiley Interscience, New Jersey, pp 49–57, 91–97
22. Deffence E (1985) Fractionation of palm oil. J Am Oil Chem Soc 62:376–385
23. Ferrari RA, Schulte E, Esteves W, Bruhl L, Mukherjee KD (1996) Minor constituents of vegetable oils during industrial processing. J Am Oil Chem Soc 73:587–592
24. Nor Aini, Che Maimon CH, Hanirah H, Zawiah S, Che Man YB (1999) Trans free vanaspati containing ternary blends of palm oil–palm stearin–palm olein and palm oil–palm stearin–palm kernel olein. J Am Oil Chem Soc 76:643–648
25. Busfield WK, Proschogo PN (1990) Thermal analysis of palm stearin by DSC. J Am Oil Chem Soc 67:171–175
26. Haryati T, Che Man YB, Swe PZ (1997) Effect of repeated heating on thermal behaviour of crude palm oil. J Am Oil Chem Soc 74:393–396
27. Sambanthamurthi R, Sundaram K, Tan Y-A (2000) Chemistry and biochemistry of palm oil. Prog Lipid Res 39:507–558
28. Verhe R, Verleyen T, Van Hoed V, De Greyt W (2006) Influence of refining vegetable oils on minor components. J Oil Palm Res Special Issue-April:168–179
29. Verleyen T, Forcades M, Verhe R, Dewettinck K, Huyghebaret A, De Greyt W (2002) Analysis of free and esterified sterols in vegetable oils. J Am Oil Chem Soc 79:117–122