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Physico-chemical characteristics and nutraceutical distribution of crude palm oil and its fractions

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SUMMARY: Crude palm oil (CRPO) was dry fractionated at 25 °C to get crude palm olein (CRPOL, 77%) and crude palm stearin (CRPS, 23%). Low and high melting crude palm stearin (LMCRPS 14.3% and HMCRPS 8.7%) were separated by further fractionation of CRPS with acetone. The physico-chemical parameters and nutraceutical distribution showed variation in different fractions. The CRPO contained 514.7 mg·Kg^{-1} of β-carotene and 82.6%, 16.1%, 12.5% and 3.1% of it was distributed in CRPOL, CRPS, LMCRPS and HMCRPS respectively. The distribution of phytosterols in fraction was 1870.2, 1996.8, 1190.9, 1290.4 and 115.4 mg·Kg^{-1} for CRPO, CRPOL, CRPS, LMCRPS and HMCRPS respectively. Total tocopherol composition was 535.5, 587.1, 308.0, 305.6 and 36.2 mg·Kg^{-1} for CRPO, CRPOL, CRPS, LMCRPS and HMCRPS respectively. The results show that the fractionation of CRPO may be helpful in the preparation of nutraceutical-rich fractions.

KEYWORDS: *Crude palm oil; Crude palm oil fractions; Nutraceuticals; Physico-chemical characteristics; Radical scavenging activity*

RESUMEN: ***Características fisicoquímicas y distribución nutracéutica de aceite de palma crudo y sus fracciones.*** Aceite de palma crudo (CRPO) fue fraccionado en seco a 25 °C para obtener oleína de palma cruda (CRPOL, 77%) y estearinas de palma cruda (CRPS, el 23%). Estearinas con bajo y alto punto de fusión (LMCRPS 14,3% y HMCRPS 8,7%) se separan por fraccionamiento adicional de CRPS con acetona. Los parámetros físico-químicos y la distribución de nutracéuticos muestra diferencias entre las fracciones. El CRPO contenía 514,7 mg·Kg^{-1} de β-caroteno y el 82,6%, 16,1%, 12,5% y 3,1% de este se distribuye en CRPOL, CRPS, LMCRPS y HMCRPS respectivamente. Los fitosteroles en las fracciones fue de: 1870,2, 1996,8, 1190.9, 1290,4 y 115,4 mg·Kg^{-1} para CRPO, CRPOL, CRPS, LMCRPS y HMCRPS respectivamente. La composición total de tocoferol fue 535,5, 587,1 308,0, 305,6 y 36,2 mg·Kg^{-1}, para CRPO, CRPOL, CRPS, LMCRPS y HMCRPS respectivamente. Los resultados mostraron que el fraccionamiento de CRPO puede ser útil en la preparación de fracciones ricas en nutracéuticos.

PALABRAS CLAVE: *Aceite de palma crudo; Actividad de captación de radicales; Características físico-químicas; Fracciones de aceite de palma crudo; Nutracéuticos*

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1. INTRODUCTION

Palm oil is obtained from the fleshy fruit mesocarp of *Elaeis guineensis,* a high oil-yielding perennial crop which has grown to be one of the most important vegetable oils due to its advantageous properties such as high productivity, low price, high oxidation stability, fatty acid composition and, finally, good plasticity at room temperature (Nor Aini *et al*., 1999; Miskandar *et al*., 2006). Palm oil contains almost equal quantities of saturated (palmitic 44% and stearic 4%) and unsaturated fatty acids (oleic acid 39% and linoleic acid 11%) (Gunstone *et al*., 2007). It consists of >90% triglycerides, 2–7% diglycerides,<1% monoglycerides and 3–4% free fatty acids and approximately 1% of minor components which include carotenoids, vitamin E (tocopherols and tocotrienols), sterols, phospholipids, glycolipids, and terpenic and aliphatic hydrocarbons, which contribute to the stability and nutritional properties of palm oil (Goh *et al*., 1985, Choo and Tay, 1985; Anderson, 1996).

Palm oil is semi-solid in consistency under ambient conditions (20–30 °C). It consists of an admixture of low melting and high melting glycerides which form a heterogeneous slurry in the liquid oil (Timms, 1985). Because of its solid nature various processes are employed to modify the properties of palm oil and to widen its commercial use. There are several modification processes: viz. hydrogenation, interesterification (chemical and enzymatic) or fractionation (dry, solvent and detergent fraction) (Rodrigues, *et al*., 2007). The hydrogenation process creates trans fatty acids which have a negative impact on health (Nor Aini *et al.* 1999). Interesterification is carried out by means of enzymes or chemicals to modify oil blends. Fractionation involves selective crystallization and filtration and is based on the differences in the melting points of TAG. Dry fractionation is the simplest and cheapest fractionation method. Here there would not be any generation of effluent, no use of chemicals and no loss in oil (Kellens *et al.*, 2007). But, a part of the liquid will always remain entrapped within the solid phase. In this case solvent fraction helps in an effective separation of olein from stearin.

Usually palm oil undergoes refining, bleaching and deodorization steps. Steam distillation is the commonly engaged purification process and operates at high temperatures (250–265 °C) under vacuum (5 mmHg). Under this condition minor components including carotenoids and tocotrienols, which are potent fat-soluble antioxidants present in crude palm oil, are degraded or stripped off and accumulate in the palm fatty acid distillate (PFAD). This mode of purification and further fractionation leads to a loss in minor components from the starting palm oil and would not be available for consumption. The final nutraceutical quality of the product must be evaluated in depth. The evaluation of nutraceutical retention in palm oil by alternate methods like solvent deacidification, enzymatic deacidification instead of conventional steam distillation is also important. Gee (2007) has made an attempt to compare the analytical characteristics of crude and refined palm oil fractions. No study so far reported the effect of fractionation on the nutracraceutical distribution of palm oil fractions. This study provides information on the physico-chemical characteristics and nutraceutical composition of different palm oil fractions. This may helpful to design a suitable deacidification process to make the nutraceuticals retained in palm oil fractions suitable for various food applications.

2. MATERIALS AND METHODS

2.1. Materials

Crude red palm oil (CRPO) extracted from the Indian grown *Elaeis guineensis* was obtained from a local palm oil industry (M/s Palm Tech India Pvt. Ltd., Mysore, India). α-Tocopherol, cholesterol, β-sitosterol, stigmasterol, stigmastanol, gallic acid, squalene, coenzyme Q_{10} and 1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co. (St Louis, MO, USA). All solvents and chemicals used were of analytical grade.

2.2. Separation of different fractions from crude red palm oil

Crude red palm oil (CRPO) fractions were collected by two different fractionation methods including dry fractionation and solvent fractionation. Dry fractionation was carried out for the initial fractionation of CRPO. Initially three different batches of CRPO obtained from the industry (20 kg each) were melted at 65 °C, equal portions of these three batches were mixed well and kept for crystallization at 25 °C for 24 h in a temperature controlled chamber. The same amount was fractionated using vacuum filtration into a liquid fraction and solid fraction. These fractions were considered as 77% of liquid olein (CRPOL) and 23% of solid stearin (CRPS). Dry fractionation cannot be used to separate the liquid fraction entrapped in the stearin fraction because of its higher viscosity, low volatility and the small differences between molecular weight and component volatilities of the stearin fraction. The solid fraction (CRPS) was further fractionated by solvent to separate residual olein at 25 °C using acetone in the ratio of 1:4 to the CRPS. The strength of separation of LMCRPS and HMCRPS

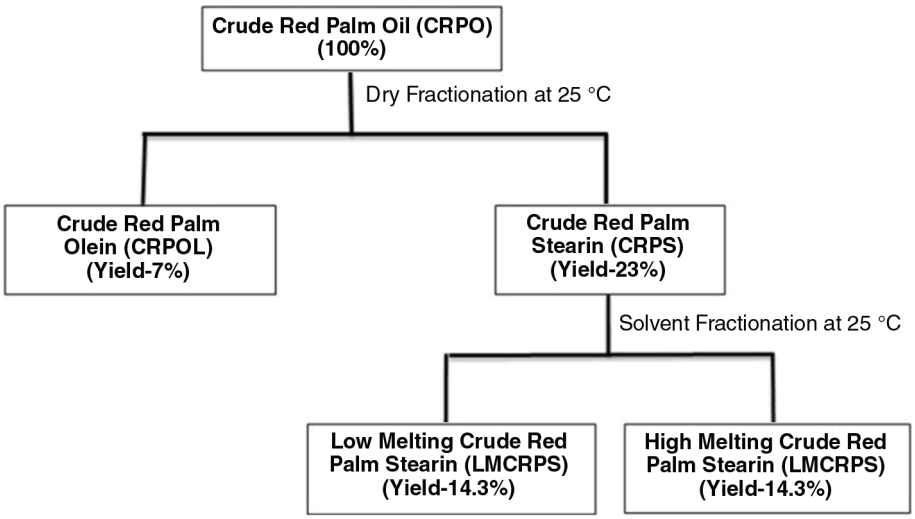

FIGURE 1. Schematic diagram for the preparation of different fractions from crude palm oil.

depends on achieving high solubility and selectivity for the high melting and low melting triglycerides present. Hence, the solvent fractionation was carried out to achieve higher solubility of low melting glycerides from CRPS. The crystallized material was separated by vacuum filtration and the solvent was evaporated using Rotavapor (Buchi Labortechnik, Swizerland) at 40 °C. The fractions obtained were low melting CRPS (LMCRPS) (14.3%) and high melting CRPS (HMCRPS) (8.7%) and used for further study (Figure 1). Both these fractions were collected and kept for 48 h at room temperature (27 °C) and its physical appearance was observed. Semisolid consistency for the liquid fraction and thick solid consistency for the solid fraction were observed. Thus, both fractions were considered as low melting crude palm stearin (LMCRPS) and high melting crude palm stearin (HMCRPS), respectively, for the liquid and solid fractions. LMCRPS are the liquid fraction physically entrapped on the surface of solid fat crystals.

2.3. Physical characteristics of crude palm oil and its different fractions

2.3.1. Color

The color of CRPO and its fractions was evaluated using a Hunter Lab Labscan XE spectrophotometer (Hunter Associates Laboratory Inc, Restone Virginia, USA). 15 mL of sample were placed in a sample cup and were used for transmittance color measurements in liquid media. Color of samples was obtained by using a 2/°C (2° observer/ illuminant C). The results were expressed as L*, a*, b*, respectively indicating lightness (0–100), green-red components and blue-yellow components.

2.3.2. Slip melting point

The slip melting point (SMP) of the different fractions was analyzed according to AOCS Official Methods (AOCS O.M. No. Cc 3-25, 1997). Samples were taken in an open capillary tube to the height of 1 cm. Samples were tempered at 10 °C for 16 h. The tubes were heated slowly in a temperature controlled water bath until the fat column rose due to hydrostatic pressure. The temperature at the rising of the fat column was expressed as SMP using the average of four replicates.

2.4. Chemical characteristics of crude palm oil and its different fractions

The free fatty acid value (FFA) was determined by AOCS O.M.No. Ca 5a-40. Oil was titrated against 0.1 N of NaOH solution in a neutralized alcohol medium using phenolphthalein as indicator and expressed as the percentage of palmitic acid. The peroxide value (PV) of the samples was determined by titration against 0.1 N of sodium thiosulphate solution in the presence of a potassium iodide solution using starch as indicator (O.M.No. Ca 8-53). The saponification value was determined by AOCS O.M.No. Cd 3-25. 5 g of samples were saponified using 50 mL of 5% ethanolic KOH solution in a conical flask connected to an air condenser and boiled until the oil was completely saponified, cooled and titrated with 0.5 N HCl using phenolphthalein as indicator. The iodine value (IV) was determined according to AOCS O.M.No. Cd 1d-92 (Wijs Method). The sample taken in carbon tetrachloride was treated with 25 mL of Wijs solution. The excess of iodine monochloride was treated with potassium iodide and liberated iodine was titrated with 0.1 N

of sodium thiosulphate solution using starch as indicator. Unsaponifiable matter was determined according to AOCS O.M.No. Ca 6a-40. 5 g of oil samples were refluxed with 5 mL of 50% KOH solution in the presence of 30 mL ethanol until the oil was completely saponified, extracted with petroleum ether, desolventized and its weight was determined. The experiments were carried out in quadruplicate and the arithmetic mean was reported.

2.5. Separation and quantification of glyceride classes

The monoacylglyceride (MAG), diacylglyceride (DAG) and Triacylglyceride (TAG) contents of the samples were estimated by using AOCS O.M.No. Cd 11c-93 (2004). A glass column (i.d. 1.8 cm; length, 30 cm) (Borosil Glass Works Ltd., Mumbai, India) was used in which a silica (100–120 mesh size) bed was prepared from the slurry of silica in petroleum ether. MAG, DAG, and TAG were eluted with a standard solvent system and the quantity of each fraction was determined gravimetrically after evaporating the solvent as per AOCS O.M.No. Cd 11c-93.

2.6. Preparation of fatty acid methyl esters and their analysis by GC

The fatty acid methyl esters (FAME) of the oil samples were prepared by transesterification using methanolic KOH, according to AOCS O.M.No. Ce 2-66. The analysis was done using a gas chromatograph (model-GC-20A, Shimadzu Corporation, Japan) equipped with an FID detector and a glass capillary column (30 m×0.25 mm), coated with poly (90% biscyanopropyl/10% cyanopropylphenyl) siloxane with a film thickness of 0.2 µm (SP-2380) (Supelco Analytical, Bellefonte, Pennsylvania, USA). The operating conditions were as follows: nitrogen flow 40 mL·min^{-1}, hydrogen flow 40mL·min^{-1}, air flow 300 mL·min^{-1}, column temperature maintained isothermal at 180 °C, injector temperature 220 °C and detector temperature 230 °C. A reference standard FAME mixture (Supelco Inc., Bellefonte, PA, USA) was analyzed under the same operating conditions to determine peak identity. The FAMEs were expressed as relative area% (AOCS O.M.No. Ce 2-66).

2.7. Triglyceride composition by HPLC

The triglyceride composition was analyzed using a Shimadzu HPLC system consisting of an LC-10A pump, fitted with a 20 µL injector pump and an RID-10A detector. Isocratic separation of the triglycerides was achieved by reverse phase HPLC on a C18 column (Discovery C18 column, 15 cm×4 mm id, 5 µm, Sigma-Aldrich, Bellefonte, PA, USA). The mobile phase was acetone: acetonitrile (70:30,v/v). TAG was calculated as a relative area percentage as per AOCS O.M.No. Ce 5b-89. TAG peaks were identified based on the theoretical carbon number described by Bland *et al.,* (1991).

2.8. Nutraceutical composition of palm oil and its fractions

2.8.1. Determination of carotene by the spectrophotometric method

The carotene content was determined by diluting 1g of melted palm oil at 65 °C to 10 mL using acetone and from this a 1 mL aliquot was further diluted to 10 mL with acetone and the absorbance was measured at 446 nm using a UV-vis spectrophotometer Shimadzu UV-1601 (Shimadzu Corporation, Kyoto, Japan) followed by calculation using the diffusion coefficient of 383 and expressed as mg·kg^{-1} oil (Chandrasekaram *et al.*, 2009).

2.8.2. Determination of tocopherol composition by HPLC

The determination of tocopherol composition by HPLC (Shimadzu) consisted of an LC-10A pump, injector fitted with 20 µL loop and Fluorescent detector. The analysis was carried out with normal phase HPLC separation on a silica column (LichroCART 250-4, Lichosorb Si60 (5 µm) 25 cm×4 mm id column, Merck KGaA, Darmstadt, Germany). The mobile phase was hexane: isopropyl alcohol (99.5:0.5,v/v) at a flow rate of 1mL·min^{-1} and detected by fluorescence at excitation and emission wavelengths of 290 nm and 330 nm respectively. Tocopherols were quantified based on peak areas with an external standard α-tocopherol. Tocotrienols were expressed as α-tocopherol equivalents as suggested by the AOCS Official Method O.M.No. Ce 8-86 (2004).

2.8.3. Estimation of phytosterols by HPLC

HPLC equipped with an LC-10A pump (Shimadzu, Tokyo, Japan), a 20 µL injection loop and a photo diode-array detector was used for the estimation of individual phytosterols in the samples. The temperature was controlled at 30±0.1 °C with a column heater. Separation was performed on a Kromasil 100 C18 5 µm column (15 cm×0.4 cm i.d.; Teknokroma, Barcelona, Spain), with 30:70 (v/v) methanol: acetonitrile as mobile phase with a flow rate of 1.2 mL·min^{-1}. The detection wavelength was 205 nm (Sánchez-Machado *et al*., 2004).

2.8.4. Determination of total phenolics in different palm oil fractions

Phenolics were extracted with methanol/water (80:20v/v) (Brenes *et al*., 2000). 5 g of samples were mixed with 1.0mL of methanol/water (80:20) and

vortexed for 2 min (twice). The mixture was centrifuged at 1080 g for 15 min and the resultant supernatant was separated. The extractions were repeated four times with the same sample with a 1.0mL portion of the solvent system. The resulting extracts were pooled together and kept in the dark till the time of analysis (Marina *et al*., 2009). The total phenolic contents of the extracts were determined by the Folin-Ciocalteu reagent method. 0.3 mL of extracts were mixed with 0.2 mL of Folin-Ciocalteu reagent and after 3 min, 1 mL of 15% Na_2CO_3 solution was added. The final volume was made up to 7 mL with de-ionized water and incubated for 45 min. The mixture was centrifuged and absorbance was measured at 745 nm in a UV-visible spectrophotometer (UV-1601, Shimadzu Corporation, Kyoto, Japan) with respect to a blank without any added phenolic extract. The total phenolic content was expressed as mg gallic acid equivalent (GAE) 100 g^{-1} of sample (Nigel *et al*., 2001).

2.8.5. Estimation of squalene by HPLC

Squalene in the samples was determined by HPLC according to Nenadis and Tsimidou (2002). Samples (0.02 g) were dissolved in 1 mL of hexane, and 20 µL of the mixture were injected into an HPLC (10A VP, Shimadzu, Kyoto, Japan) equipped with a UV-detector (SPD-10AV VP, Shimadzu). The analysis was carried out with isocratic separation on a C-18 column, 10 µm, u-Bondapack, (4.6×300 mm; Millipore, Milford, MA) with a mobile phase consisting of acetone:acetonitrile (40/60, v/v) at flow rate of 1 mL·min^{-1}. The analysis as carried out at 25 °C and the UV-detector was set at 208 nm (Nenadis and Tsimidou, 2002). Identification was confirmed through spiking with squalene and quantified using a standard curve.

2.8.6. Determination of coenzyme Q_{10} by HPLC

The determination of the content of the coenzyme Q_{10} was carried out by HPLC (Shimadzu) equipped with (10A VP, Shimadzu, Kyoto, Japan) a UV-detector (SPD-10AV VP, Shimadzu) coupled to a 20 µL loop injector. The isocratic separation of samples was achieved on a C-18 column, 10 µm, u-Bondapack, (4.6×300 mm; Millipore, Milford, MA) using methanol/*n*-hexane/2-propanol (80:15:5, v/v/v) as the mobile phase at a flow rate of 1 mL·min^{-1}. The detection wavelength was set at 275nm (Pirjo and Jorma, 2001). Samples were quantified using a standard curve.

2.9. Determination of DPPH radical scavenging activity (RSA)

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of the free radicalscavenging activity of the samples. Sample sizes of 5, 10, 25, 50, and 75 mg were mixed to an equal volume of 10^{-4} M toluenic solution of DPPH (4mL). The mixtures were incubated at room temperature for 30 min in the dark and absorbance was read at 517nm against a blank. The RSA was calculated using the following formula:

$$\% \text{ radical scavenging activity} = \{(A_{control} - A_{sample})/A_{control}\} \times 100.$$

Where, $A_{control}$ is the absorbance of the control without extract, and A_{sample} is the absorbance of the reaction mixture. The RSA was expressed as IC_{50} and was calculated using a plot of percent radical-scavenging activity against concentration (mg·mL^{-1}) to determine the concentration of extract necessary to reduce DPPH by 50% (Shimada *et al*., 1992).

2.10. Statistical analysis

All data were expressed as the mean±standard deviation of the quadruplicate analysis. The Tukeys-Kramer Multiple Comparison Test was used to calculate significance differences using the statistical package, GraphPad Instat Demo [DATA-SET.ISD]. Statistical significance was declared at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Physical properties of different palm oil fractions

Palm oil is light yellow to orange-red in color, depending on its carotenoid content. Rossi *et al*., 2001 reported the L*a*b* value of Indonesian crude palm oil as 39.03, 47.35 and 67.26, respectively (Rossi *et al*., 2001). The CRPO showed a higher L* value (43.9), lower a* (28.7) and lower b* value (39.3) as compared to Indonesian crude palm oil. The higher L* value of HMCRPS (86.2) among the fractions may be due to a loss in color compounds like carotenoids. The chromatic color compound a* indicates +red to −green. The increased positive value of a* indicates an increased value of red. The redness of samples correlated (r=0.9903) well with the carotenoid content. The color component b* indicates+yellow to −blue. The yellowish color is higher in CRPS (55.4) and lower with HMCRPS (10.7) (Table 1).

The maximum limit of slip melting point (SMP) of palm olein is 24 °C and a further decrease in SMP makes the oil more clear, but this will increase the processing cost (Miskandar *et al*., 2005). The present CRPOL obtained through the dry fractionation at 25 °C showed an SMP of 23 °C, which is within the maximum limit (24 °C) reported by Miskandar *et al.* (2005). The CRPS fraction has a higher SMP (52 °C) due to the presence of higher melting glycerides as compared to CRPOL. Further solvent fractionation helped in separating higher

Table 1. Physico-chemical characteristics of different fractions prepared from crude red palm oil

Parameters	CRPO	CRPOL	CRPS	LMCRPS	HMCRPS
Color					
L*	43.9±0.0^{a}	49.8±0.1^{b}	55.9±0.0^{c}	48.0±0.01^{d}	86.2±0.01^{e}
a*	28.7±0.1^{a}	36.5±0.1^{b}	21.2±0.01^{c}	28.4±0.02^{d}	0.8±0.1^{e}
b*	39.3±0.7^{a}	49.7±0.1^{b}	55.4±0.03^{c}	47.0±0.04^{d}	10.7±0.02^{e}
SMP (°C)	25.5±0.18^{a}	23.0±0.18^{b}	52.0±0.35^{c}	36.0±0.18^{d}	61.0±0.35^{e}
FFA (%)	7.16±0.04^{a}	7.36±0.16^{a}	4.98±0.24^{b}	6.26±0.17^{c}	0.87±0.00^{d}
PV (meq $O_2 \cdot Kg^{-1}$)	5.97±0.02^{a}	6.85±0.04^{b}	5.97±0.02^{a}	6.15±0.04^{c}	trace
SV (mg $KOH \cdot g^{-1}$)	198±1.40^{a}	197±0.57^{a}	201±1.3^{b}	198±0.42^{a}	203±0.42^{b}
IV (g $I_2 \cdot 100g^{-1}$)	50.6±0.38^{a}	53.4±0.33^{b}	34.2±0.25^{c}	45.8±0.38^{d}	16.0±0.34^{e}
USM (%)	0.54±0.04^{a}	0.63±0.02^{a}	0.24±0.04^{b}	0.42±0.04^{c}	0.18±0.07^{b}
MAG (%)	3.87±0.08^{a}	3.75±0.12^{ab}	3.73±0.24^{ab}	4.21±0.21^{a}	3.00±0.06^{c}
DAG (%)	8.83±0.10^{a}	10.54±0.16^{b}	8.24±0.01^{c}	11.25±0.10^{d}	5.59±0.12^{e}
TAG (%)	80.14±0.12^{a}	78.56±0.08^{b}	83.40±0.12^{c}	78.04±0.24^{d}	90.54±0.14^{e}

All values are average±standard deviation. N=4. Values in the same row with a different superscript indicate significant difference at $p<0.05$. CRPO=Crude Red Palm Oil, CRPOL=Crude Red Palm Olein, CRPS=Crude Red Palm Stearin, LMCRPS=Low Melting Crude Red Palm Stearin, HMCRPS=High Melting Crude Red Palm Stearin.

melting glycerides and lower melting glycerides from CRPS. Separation efficiency was reflected in SMP and showed 36 °C for LMCRPS and 61 °C for HMCRPS. CRPS has limited food application because of its higher melting point. Hence, solvent fractionation of CRPS may help to widen its application in the food industry by the utilization of LMCRPS.

3.2. Chemical properties of different palm oil fractions

Choon-Hui *et al*. (2009) have reported the free fatty acid value (FFA) of crude palm oil to range from 2.3% to 6.7% and poor or lengthy storage of palm fruits will lead to a considerable increase in FFA of palm oil (Choon-Hui *et al*., 2009). The higher FFA of CRPO was mostly due to the action of microbial lipase present in palm fruit. Primary fractions viz CRPOL and CRPS contain 7.36 and 4.98%, respectively. Secondary fractions viz LMCRPS and HMCRPS contain 6.23 and 0.87%, respectively. All fractions except CRPOL have FFA contents less than that of the starting CRPO (7.16%). The FFA contents of all the fractions indicate that FFA was distributed in higher levels in the liquid fraction than in the solid fraction. Fractionation helps to prepare the fraction with lower FFA than the starting CRPO. Here HMCRPS showed the lowest FFA (0.87%) among all the fractions.

The amount of hydroperoxides in oil can be indexed by the peroxide value (PV). The oil with a large amount of unsaturated fatty acids is more prone to oxidation than oil with high levels of saturated fatty acids. The initial CRPO showed 5.97 $meqO_2 \cdot Kg^{-1}$ of PV. Primary fractionation products showed a PV of 6.85 and 5.97 $meqO_2 \cdot Kg^{-1}$ respectively for CRPOL and CRPS. Secondary fractionation products showed 6.15 $meqO_2 \cdot Kg^{-1}$ for LMCRPS and below detection limit (<0.5 $meqO_2 \cdot Kg^{-1}$) for HMCRPS. The high levels of PV in CRPOL (6.85 $meqO_2 \cdot Kg^{-1}$) and LMCRPS (6.15 $meqO_2 \cdot Kg^{-1}$) was mostly due to a higher level of distribution of hydroperoxides in these fractions than in the other fractions and may be due to the formation of new hydroperoxides during the primary and secondary fractionation processes. The PV of HMCRPS indicated that the fractionation process can be used for the preparation of fractions with low or no hydro peroxide.

The saponification value determines the physicochemical properties of an oil and indicates the average molecular weight of the fatty acids present in the oil. The analysis of SV shows that HMCRPS (203 mg $KOH \cdot g^{-1}$) had the highest value while CRPOL (197 mg $KOH \cdot g^{-1}$) had lowest among all the samples. The SV showed an increase from liquid CRPOL (197 mg $KOH \cdot g^{-1}$) to solid HMCRPS (203 mg $KOH \cdot g^{-1}$) indicating that changes in the glyceride composition of each sample had occurred. Here the values showed an increase of SV from olein to stearin, indicating a variation in average molecular weight.

The fractionation of CRPO modifies the physicochemical characteristics of fractions by changing the iodine value (IV) and saturated/unsaturated fatty acid ratio. IV is an indicator for measuring the unsaturation of oils and it can be used to define the quality and functionality of the fractions.

The results showed that the most unsaturated fraction was CRPOL (53.4). The least unsaturation was shown by HMCRPS (16.0). The CRPO showed an IV of 50.6 and the fractionation of CRPO provided two fractions with IV of 53.4 and 34.2. The variation in the IV of the two fractions indicates significant levels of separation of saturated fatty acids from the starting material. Similarly, the fractionation of CRPS IV of 34.2 provided two different fractions having IV of 45.84 and 16.02 which indicates the separation of highly saturated glycerides from the CRPS.

The unsaponifiable matter of crude palm oil comprises mainly carotenoids, tocopherols, tocotrienols, phytosterols, squalene and the coenzyme Q. The results showed 0.54% of unsaponifiable matter in the starting palm oil. CRPOL showed enrichment in unsaponifiable matter (0.63%). CRPS showed 0.24% of unsaponifiable matter and the further fractionation showed an enrichment of unsaponifiable matter in LMRPS (0.42%). HMCRPS showed the least amount (0.18%) of unsaponifiable matter as compared to other fractions. The unsaponifiable matter in the samples showed that the level of incorporation of unsaponifiable matter depends on the fluidity.

3.3. Glyceride classes

The content of acylglycerols is an important determinant of oil quality. They will affect the physical properties of oil and can cause cloudiness in oil even at less than 10% of total concentration. DAG, MAG and FFA are the metabolites in the biosynthesis of TAG and the products of lipolytic activity. MAG and DAG are present in significant amounts in palm oil. CRPO consists of 0.21–0.34% MAG. In the present study CRPO showed MAG of 3.78%. The MAG level was higher in LMCRPS (4.21%) as compared to other fractions. A total DAG concentration in CRPO ranging from 5.3 to 7.7% has been reported in the literature (Jacobsberg and Oh, 1976). The DAG level of CRPO showed 9.09%. The lowest DAG level was observed for HMCRPS (3.93%) and the highest level was for LMCRPS (11.25%). The result showed that DAG was more soluble with olein than with stearin. The levels of MAG and DAG in turn affected the level of TAG in the samples.

3.4. Fatty acid composition

The fatty acid composition of the CRPO showed that palmitic acid (41.8%), oleic acid (37.4%) and linoleic acid (14.1%) were the major fatty acids in CRPO. The minor fatty acids found were stearic acid (3.5%), myristic acid (1.6%), lauric acid (1.2%), caprylic acid (0.3%) and caproic acid (0.2%) (Table 2). These fatty acids are distributed differently in each fraction. The palmitic acid level was 40.9% in CRPOL, 55.7% in CRPS, 43.7% in LMCRPS and 79.7% in HMCRPS. Similarly the level of stearic acid was the lowest with CRPOL (3.3%) and the highest with HMCRPS (5.7%). The oleic acid level showed significant difference among the samples. CRPO showed oleic acid of 37.2%. Through fractionation it was enriched to 42.3% in CRPOL. Similarly, the oleic acid level was further enriched in LMCRPS (40.2%) through the fractionation of CRPS (30.6%). The compositions of palmitic acid and oleic acid in CRPO, CRPOL and CRPS were comparable with the values reported by Gee (2007) which showed 40.9%–47.5%, 36.8%–43.2% and 49.8%–68.1% of palmitic acid and 36.4%–41.2%, 39.8%–44.6% and 20.4%–34.4% of oleic acid, respectively, in palm oil, palm olein and palm stearin. CRPOL showed low saturation (46.1%) and HMCRPS showed high saturation (86.9%) among the samples. The result showed monounsaturated fatty acids were present in the decreasing order of: CRPOL>LMCRPS>CRPO>CRPS>HMCRPS.

3.5. Triglycerides

The TAG composition of CRPO and its primary and secondary fractions were analyzed. The main TAG of CRPO was POP (25.5%), PPP (20.8%), POO (14.0%), PLP/MOP (7.8%) and POL (6.7%, where P is palmitic acid, O is oleic acid, L is linoleic acid and M is myristic acid. The minor TAGs in CRPO include: PPS (4.8%), SOP (4.7%), OOO (2.3%), SOO (2.0%), MOL (2.0%), OOL (1.2%) and other TAGs with 0.6% (Table 3). The results showed that fractionation has significantly affected the composition of various TAGs in each fraction. This in turn altered the physico-chemical properties of the fractions. The highest level of TAG (POP) was distributed in almost similar levels in CRPOL (25.4%), CRPS (23.7%) and LMCRPS (28.2%) while in HMCRPS the level of POP became the least (6.3%) when compared to other samples (Figure 2).

3.6. Nutraceutical composition of different fractions

The results showed that the carotenoid content of crude palm oil was 514.7 $mg \cdot Kg^{-1}$ and comparatively higher than the carotene content of Indonesian crude palm oil (456 $mg \cdot Kg^{-1}$) (Rossi *et al.*, 2001). The dry fractionation of CRPO has helped to enrich the carotenoid level in CRPOL (569 $mg \cdot Kg^{-1}$). The 82.2% of carotenoids were distributed in CRPOL and 16.1% (338 $mg \cdot Kg^{-1}$) were distributed in CRPS. The further solvent fractionation of CRPS showed 12.5% (431 $mg \cdot Kg^{-1}$) of carotenoids present in CRPO distributed in LMCRPS and 3.1% (8.6 $mg \cdot Kg^{-1}$) were distributed in HMCRPS. The dry fractionation of CRPS helped to enrich the carotenoid level from 338 $mg \cdot Kg^{-1}$ to 431 $mg \cdot Kg^{-1}$ in LMCRPS (Table 4).

Table 2. Fatty acid composition (%) of crude palm oil and its fractions

Fatty acids	CRPO	CRPOL	CRPS	LMCRPS	HMCRPS
Caprylic (C8:0)	0.3±0.04[a]	0.1±0.00[a]	nd	nd	nd
Caproic (C10:0)	0.2±0.01[a]	0.1±0.00[a]	nd	nd	nd
Lauric (C12:0)	1.2±0.04[a]	0.5±0.02[b]	0.2±0.01[c]	0.3±0.01[d]	nd
Myristic (C14:0)	1.6±0.02[a]	1.2±0.02[b]	1.4±0.02[c]	1.4±0.02[c]	1.5±0.04[d]
Palmitic (C16:0)	41.8±0.35[a]	40.9±0.73[a]	55.7±0.35[b]	43.7±0.73[c]	79.7±1.20[d]
Stearic (C18:0)	3.5±0.08[a]	3.3±0.14[a]	3.9±0.28[b]	3.7±0.03[ab]	5.7±0.02[c]
Oleic (C18:1)	37.4±0.40[a]	42.3±0.21[b]	30.6±0.73[c]	40.2±0.35[d]	10.1±0.06[e]
Linoleic (C18:2)	14.1±0.06[a]	11.6±0.03[b]	8.2±0.03[c]	10.8±0.04[d]	3.0±0.04[e]
SAFA	48.6	46.1	61.2	49.1	86.9
MUFA	37.4	42.3	30.6	40.2	10.1
PUFA	14.1	11.6	8.2	10.8	3.0

All values are average±standard deviation. N=4. Values in the same row with different superscript indicate significant difference at $p<0.05$. CRPO=Crude Red Palm Oil, CRPOL=Crude Red Palm Olein, CRPS=Crude Red Palm Stearin, LMCRPS=Low Melting Crude Red Palm Stearin, HMCRPS=High Melting Crude Red Palm Stearin.

Tocotrienols are the main features of palm oil as compared to other vegetable oils, no other vegetable oil except rice bran oil contains tocotrienols in significant amounts. It is reported that the 70–80% of total vitamin E is constituted by tocotrienols (Chiew *et al.,* 2007). Indonesian crude palm oil showed 25% of tocopherols and 75% of tocotrienols according to Rossi *et al.*, 2001. The level of tocotrienols in crude palm oil showed 90.1% of the total vitamin E, which indicates the fact that the ratio of tocotrienols to tocopherols (90:10) was high compared to the vitamin E composition of Indonesian crude

Table 3. Triglyceride profile of crude palm oil and its fractions

TAG	TCN	CRPO (%)	CRPOL (%)	CRPS (%)	LMCRPS (%)	HMCRPS (%)
MOL	42.7	2.0	2.7	1.2	2.9	nd
MLP/MOM	43.3/43.4	0.4	0.6	2.2	0.7	0.2
OOL	44.1	1.2	2.2	0.4	1.9	0.2
POL	44.7	6.7	9.9	1.6	10.6	1.0
PLP/MOP	45.3/45.4	7.8	8.5	6.9	9.9	1.5
MPP	46.0	1.3	0.1	7.3	0.2	2.7
OOO	46.2	2.3	3.6	3.4	3.8	1.5
POO	46.8	14.0	20.6	14.4	23.7	2.2
POP	47.4	25.5	25.4	23.7	28.2	6.3
PPP	48.0	20.8	1.6	15.2	1.5	67.3
StOO	48.8	2.0	3.2	2.6	2.8	0.5
StOP	49.4	4.7	6.2	5.7	5.5	0.8
PPSt	50.0	4.8	0.3	3.3	0.3	13.8
StOSt	51.4	0.8	8.3	7.0	0.2	0.5
PStSt	52.0	0.6	0.0	0.4	2.2	1.4
Others	–	0.5	2.3	0.6	nd	nd
Σ UUU	–	3.5	5.8	3.8	5.7	1.7
Σ SUU	–	24.7	36.3	19.8	40.1	3.6
Σ SSU	–	39.2	49.0	45.5	44.4	9.3
Σ SSS	–	27.5	2.1	26.3	4.2	85.1

Nd=not detected. M=Myristic, O=oleic, L=linoleic, P=palmitic, St=stearic, S=saturated, U=unsaturated. TCN=Theoretical Carbon Number [(ECN-(0.7×L)-(0.6×O], ECN=Equivalent Carbon number [CN-(2×ND), L=linoleic acid, O=Oleic, CN=Carbon Number, ND=Number of Double bond, CRPO=Crude Red Palm Oil, CRPOL=Crude Red Palm Olein, CRPS=Crude Red Palm Stearin, LMCRPS=Low Melting Crude Red Palm Stearin, HMCRPS=High Melting Crude Red Palm Stearin.

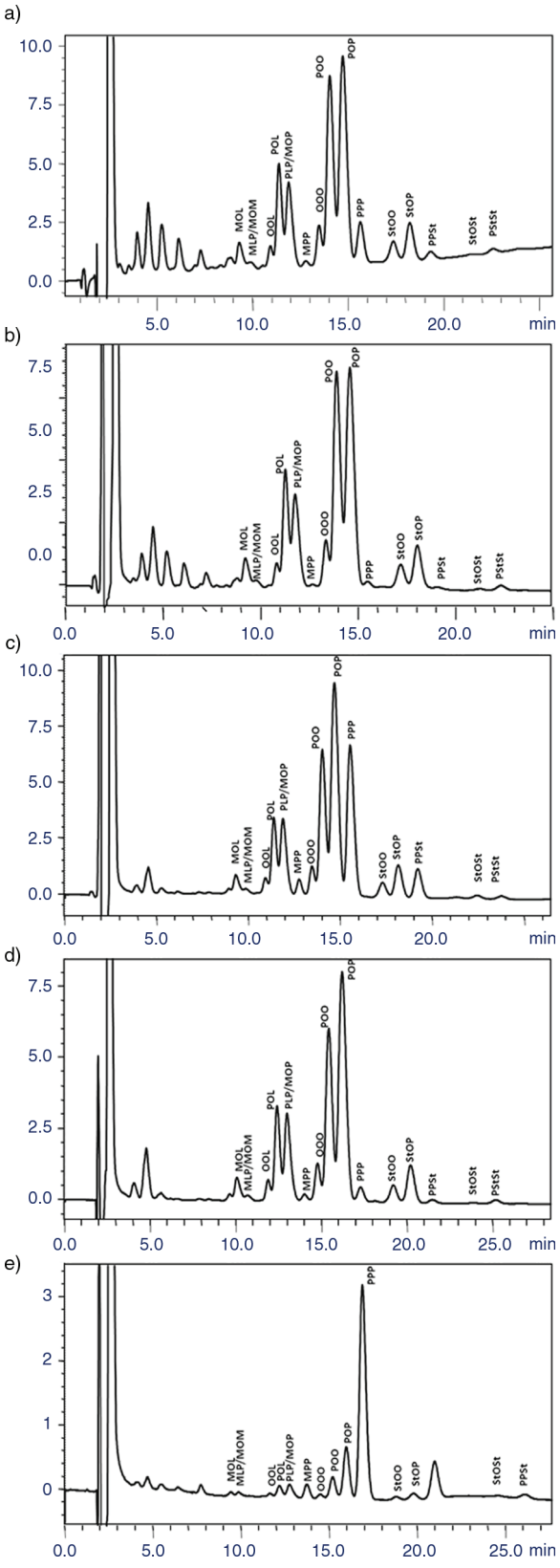

FIGURE 2. Triglyceride profile of a) crude red palm oil (CRPO), b) red palm olein (CRPOL), c) red palm stearin (CRPS), d) low melting red palm stearin (LMCRPS) and e) high melting red palm stearin (HMCRPS).

palm oil (75:25). The tocotrienol level for CRPOL was 88.4%, for CRPS 87.0%, for LMCRPS 87.5% and for HMCRPS 91.7%. The α, γ and δ tocotrienols are the most prominent tocotrienols in palm oil. The δ-tocotrienol was the highest among other tocotrienols, and ranged from 21 mg·Kg^{-1} for HMCRPS to 236 mg·Kg^{-1} for CRPOL (Table 4). The level of γ-tocotrienols ranged from 7 mg·Kg^{-1} for HMCRPS to 175 mg·Kg^{-1} for CRPOL. The level of α-tocotrienols ranged from 5 mg·Kg^{-1} for HMCRPS to 107 mg·Kg^{-1} for CRPOL. The fractionation of CRPO helped to enrich the total vitamin E in CRPOL and mostly due to the solubility of vitamin E in the liquid fraction as compared to solid fractions (Sakina and Gopala Krishna, 2007). The fractionation of CRPS did not help to enrich these components in its fractions. HMCRPS contains the least amount of vitamin E as compared to other fractions. The vitamin E content of LMCRPS was almost similar to the CRPS and there was no significant enrichment in this fraction, indicating a loss in vitamin E during solvent fractionation.

Sterols make up a considerable portion of the unsaponifiable matter in oil. The total sterol content in CRPO is around 500 mg·kg^{-1}. β-Sitosterol is the most abundant sterol (up to 60%). Campesterol, stigmasterol and cholesterol were observed in lower quantities. The following phytosterols were analyzed: cholesterol, stigmasterol, β-sitosterol and stigmastanol. The results showed that cholesterol was present in the smallest amount (19.9 mg·Kg^{-1}) in CRPO. Its distribution in the different fractions of CRPO was 21.2, 16.1, 17.0 and 5.1 mg·Kg^{-1}, respectively for CRPOL, CRPS, LMCRPS and HMCRPS. The Stigmasterol level of the samples showed that there was no significant ($p \geq 0.05$) difference in CRPO (69.0 mg·Kg^{-1}) or in CRPOL (72.4 mg·Kg^{-1}). Similarly CRPS (44.2 mg·Kg^{-1}) and LMCRPS (47.1 mg·Kg^{-1}) showed no significant difference in their stigmasterol content. The β-sitosterol level showed 128.1 mg·Kg^{-1} in CRPO and 131.0 mg·Kg^{-1} for CRPOL and there was no significant difference ($P \geq 0.05$) between CRPO and CRPOL. The fraction CRPS showed 80.3 mg·Kg^{-1} and the further fractionation distributed β-sitosterol at 9.8 mg·Kg^{-1} to LMCRPS and 5.1 mg·Kg^{-1} to HMCRPS. Stigmastanol showed its highest level (1643.2 mg·Kg^{-1}) in CRPO and its fractions as compared to other phytosterols. CRPOL showed enrichment in stigmasterol (1772.2 mg·Kg^{-1}) as compared to CRPS (1050.3 mg·Kg^{-1}). Further fractionation showed an enrichment of stigmasterol in LMCRPS (1128.3 mg·Kg^{-1}) as compared to HMCRPS (101.2 mg·Kg^{-1}).

Squalene is a minor constituent of palm oil. It has been reported that the squalene content of CRPO ranges from 200–500 mg·Kg^{-1} (Kellens *et al.*, 2007). In this study CRPO showed 299.3 mg·Kg^{-1} of squalene. It was highest in CRPOL (360.5 mg·Kg^{-1}) and lowest in CRPS (16. 4 mg·Kg^{-1}). Squalene was

TABLE 4. Nutraceutical composition of crude palm oil and its fractions

Minor components (mg·Kg^{-1})	CRPO	CRPOL	CRPS	LMCRPS	HMCRPS
β-Carotene	514.7±7.34^{a}	569.4±9.16^{b}	338.0±0.93^{c}	431.5±9.79^{d}	8.6±0.05^{e}
Cholesterol	19.9±0.05^{a}	21.2±0.07^{a}	16.1±0.04^{a}	17.0±0.45^{ab}	5.1±0.22^{b}
Stigmasterol	69.0±0.24^{a}	72.4±0.28^{a}	44.2±0.14^{b}	47.1±1.26^{b}	4.0±0.17^{c}
Betasitosterol	128.1±0.62^{a}	131.0±0.49^{a}	80.3±0.15^{b}	98.0±0.75^{c}	5.1±0.10^{d}
Stigmastanol	1643.2±2.62^{a}	1772.2±2.02^{b}	1050.3±0.60^{c}	1128.3±1.2^{d}	101.2±0.1^{e}
Σ Phytosterols	1870.2	1996.8	1190.9	1290.4	115.4
α-Tocopherol	50.0±0.71^{a}	63.1±0.14^{b}	38.3±0.35^{c}	37.1±0.35^{d}	3.0±0.21^{e}
α-Tocotrienol	94.2±0.14^{a}	107.3±0.35^{b}	69.0±0.35^{c}	59.2±0.35^{d}	5.1±10^{e}
β+γ-Tocopherol	3.0±0.07^{a}	5.1±014^{b}	2.0±0.03^{c}	1.00±0.02^{d}	nd
β+γ-Tocotrienol	168.3±1.41^{a}	175.4±1.41^{b}	55.1±0.35^{c}	56.2±0.35^{c}	7.0±0.05^{d}
δ-Tocotrienols	220.4±1.41^{a}	236.2±1.41^{b}	143.6±0.70^{c}	152.3±0.70^{d}	21.1±0.10^{e}
Σ Tocopherol	53.0	68.2	40.3	38.1	3.0
Σ Tocotrienols	482.5	518.9	267.7	267.5	33.2
Tocols	535.5	587.1	308.0	305.6	36.2
Phenolics	84.3±0.9^{a}	87.2±2.1^{ab}	74.6±9.3^{a}	78.2±4.1^{ab}	nd
Squalene	299.4±24.3^{a}	360.5±25.4^{b}	16.4±0.5^{c}	19.2±4.3^{c}	nd
Coenzyme Q_{10}	40.2±0.11^{a}	80.4±0.99^{b}	10.1±0.25^{c}	16.1±0.28^{d}	nd

All values are average±standard deviation. N=4. Values in the same row with different superscript indicate significant difference at p<0.05. nd=not detected. CRPO=Crude Red Palm Oil, CRPOL=Crude Red Palm Olein, CRPS=Crude Red Palm Stearin, LMCRPS=Low Melting Crude Red Palm Stearin, HMCRPS=High Melting Crude Red Palm Stearin.

not detected in HMCRPS mostly due to the fact that the distribution of sqalene depends on the liquid nature of CRPOL. The CRPOL consists of 93% of total squalene present in the CRPO. The result showed that the distribution of squalene in stearin fraction was less (16.4 mg·Kg^{-1}) with CRPS or nil with HMCRPS. The fractionation of CRPS (16.4 mg·Kg^{-1}) showed a further enrichment of squalene (19.2 mg·Kg^{-1}) in the LMCRPS.

Ubiquinone, a fat soluble nutrient commonly known as coenzyme, is present in CRPO in the range of 10–80 mg·Kg^{-1}. The level of the coenzyme Q_{10} in CRPO was 40 mg·Kg^{-1} and it was enriched in CRPOL (80 mg·Kg^{-1}) after dry fractionation. CRPS contains 10 mg·Kg^{-1} and further fractionation showed enrichment up to 16.1 mg·Kg^{-1} in LMCRPS. This result is indicative of the fact that the distribution of the coenzyme Q_{10} increases with fluidity of the fractions.

The palm fruit is a rich source of water soluble phenolics. Most of these phenolics are removed along with the waste stream during the milling process to extract the oil from the palm fruit. It has been reported that the total phenolic content of CRPO was 91.0 mg·Kg^{-1} of oil (Szydlowska-Czerniak, *et al.*, 2011). The total phenolic content of CRPO was 84.3 mg·Kg^{-1} of oil. The fractionation of CRPO showed the distribution of phenoics in CRPOL and CRPS, respectively, as 87.2 mg·Kg^{-1} and 74.6 mg·Kg^{-1}. MCRPS showed 78.2 mg·Kg^{-1} after the fractionation of CRPS (74.6 mg·Kg^{-1}). HMCRPS did not show the presence of phenolic compounds.

3.7. Radical scavenging activities of different palm oil fractions

The DPPH radical was used to evaluate the free radical scavenging properties of different fractions of CRPO. The samples with lower IC_{50} indicate stronger RSA. CRPOL, after 30 min of reaction with DPPH, exhibited greater RSA (IC_{50}=19.2 mg·mL^{-1}) than CRPO (IC_{50}=20.9 mg·mL^{-1}) and other fractions. CRPS showed an IC_{50} value of 30.7 mg·mL^{-1}, which indicates that the presence of antioxidants

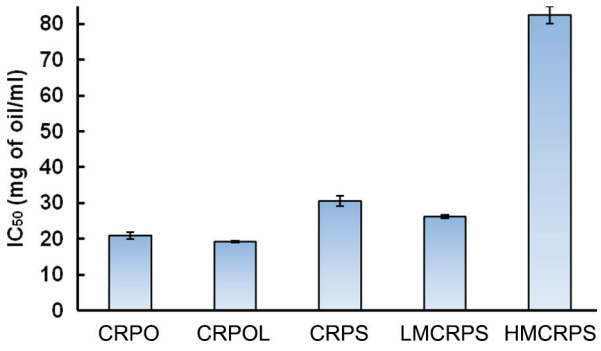

FIGURE 3. Radical scavenging activity of different fractions of crude red palm oil.

is in smaller amounts as compared to CRPOL (19 mg·mL^{-1}). LMCRPS and HMCRPS showed an IC50 value of 26 mg·mL^{-1} and 83 mg·mL^{-1} respectively. This indicates the enrichment of antioxidants in LMCRPS during the solvent fractionation of CRPS. The variation in RSA among the samples indicates a distribution of nutraceuticals to a varying extent in the different fractions. With regard to the IC_{50} value, the effectiveness of RSA in descending order was CRPOL>CRPO>LMCRPS>CRPS>HMCRPS (Figure 3).

4. CONCLUSION

Commercial purification steps operate with crude semisolid palm oil at high temperatures. Purified palm oil undergoes fractionation to provide different functional properties to foods. This way of processing leads to a nutraceutical loss especially in carotenoids, to a greater extent. This nutraceutical loss can be controlled by modifying de-acidification methods. The fractionation of crude palm oil based on the melting characteristics of triglycerides provides fractions with different properties. The present study reveals the effect of fractionation on the nutraceutical distribution in different fractions of crude palm oil. The fractions ranged from liquid to solid in their physical state. The information on the physico-chemical characteristics and nutraceutical distribution of different fractions helps in designing suitable de-acidification methods including enzymatic de-acidification and low temperature (room temperature) extraction of FFA using a solvent to retain the maximum of these heat-sensitive nutraceuticals in fractions. The de-acidification limitations associated with CRPOL, CRPS and LMCRPS to meet the standard quality and the quality parameters indicates that HMCRPS is not necessary for purification. The retention of nutraceuticals in the crude palm oil fractions make them useful as a functional food to provide nutraceuticals to consumers.

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