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Comparative study of physicochemical properties, in vitro digestion and gastrointestinal absorption of medium chain fatty acid rich rice bran and rice bran oil

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Keywords: Medium chain fatty acids, Rice bran oil, Physicochemical analysis, in vitro digestion study, in vivo absorption study.

Abstract

In our present study, we worked on the preparation of different medium chain fatty acid rich rice bran oils and comparison of their *in vitro digestion study and in vivo* intestinal absorption in the single-pass perfusion rat model. The physicochemical properties of the oils were also analyzed. Medium chain fatty acids have a better absorption capacity in comparison to long chain fatty acids. Medium chain fatty acid rich rice bran oil was prepared by enzyme catalysis technique which resulted in substitution of long chain fatty acids in rice bran oil with medium chain fatty acids. *In vitro* digestion study showed that medium chain fatty acid rich rice bran oil was better digested in comparison to native rice bran oil. *In situ* absorption efficiency of the oils was measured in laboratory acclimatized adult Sprague–Dawley rats. A comparative study of lipid absorption from medium chain fatty acid rich rice bran oil and native rice bran oil of similar dilution level, in cannulated small intestine of rats, at time gradient, has been done. Subsequent analysis (e.g. percent volume absorption, percent lipid absorption) have shown that the medium chain fatty acid rich rice bran oil has significantly enhanced the absorption of lipids from the emulsion system, in the small intestine of the rats. This finding about absorption of medium chain fatty acid rich rice bran oil could be useful for treating the infants, patients with low absorption or reduced absorption capacity

INTRODUCTION

Medium chain triglycerides (MCTs) are a family of triglyceride, composed mainly of caprylic and capric fatty acids with a minor contribution of caproic and lauric fatty acids. The concept of MCTs was introduced into clinical nutrition in the 1950s for the dietary treatment of malabsorption syndromes because of their rapid absorption and solubility (Seaton et al, 1986). MCT is mostly used to treat malabsorption states and the rationale for the use of MCT on malabsorption states is related to the circumstance that the small molecules of medium chain fatty acids (MCFAs) are more easily hydrolyzed by pancreatic lipase and is thus more rapidly absorbed (Kaunitz, 2001). The rationale for the use of MCT is based on differences in digestion, absorption, transport and catabolism between MCT and long chain triglycerides (LCT). Bile salts and micelle formation are not required for dispersion or absorption of MCT. MCT are transported across the mucosal more rapidly than LCT. MCT donot enter the lymph system and are transported through the portal venous system as albumin-bound free fatty acids. They are incorporated into chylomicrons and therefore do not require lipoprotein lipase for oxidation (Babayan, 1981). It has been observed that in comparison to LCT there is decrease in food intake in case of MCT consumption. Therefore replacing dietary LCT with MCT can increase weight maintenance programme. MCT can be used for clinical purposes such as the treatment of disorders of lipid absorption and as an energy source for enteral and parenteral nutrition (Bell *et al*., 1991).

Rice bran oil is a widely used cooking oil in Asian countries. It is well known for the presence of phytochemicals and for its stability as frying oil. Gamma-oryzanol is the important phytochemical

present only in RBO which exhibits antioxidant properties including free radical scavenging and lipoperoxidation prevention. It is also reported to be hypolipidemic (Akiyama *et al*., 2005).

MCT rich mustard oil has been proved to contribute towards protection against different lifestyle diseases in our previous studies. The food intake, growth of animals and lipid content of mesentry showed that capric acid rich mustard oil has anti-obese properties in comparison to mustard oil. Mustard oil enriched with capric acid significantly lowered plasma and liver lipids which are high risk factors of cardiovascular diseases (Sengupta and Ghosh, 2011). Consumption of capric acid rich mustard oil improves the haematological and histological conditions which were disturbed due to hypercholesterolemia. It seems to produce an inhibitory effect on platelet aggregation thus reducing the chances of vascular diseases and finally atherosclerosis by reducing the interaction between platelets and vessel wall (Sengupta and Ghosh, 2010). In another study it was observed that the deformity and fragility of erythrocyte membrane caused by cholesterol rich blood was partially reversed by capric acid rich mustard oil by virtue of their ability to lower the extent of hypercholesterolemia (Sengupta and Ghosh, 2011a). On the basis of some other data it was proposed that dietary supplementation with capric acid might benefit humans leading to improved antioxidant defenses in individuals with hypercholesterolemia and thereby lowering atherosclerosis risk ((Sengupta and Ghosh, 2012)

The in vitro digestion of lipids and their associated health effects is the subject of extensive research (Armand, 2007) . The consumption of structured lipid containing MCFA has shown to offer various health benefits as mentioned above. The behavior of those structured lipids during digestion has been poorly studied. There is also insufficient data regarding the absorption study of structured lipid containing MCFA. In the present study we have utilized single-pass perfusion method in rat model to compare the intestinal absorption of RBO and MCFA rich RBOs. The study also encompasses the in vitro intestinal digestion study of MCFA rich RBOs in comparison to native RBO. The analysis of the physico-chemical properties of MCFA rich RBOs is also under the scope of this study.

MATERIALS AND METHODS

Materials:

Refined rice bran oil was procured from the market and the acid value was checked. Caprylic acid (C8:0), Capric acid (C10:0) and Lauric acid (C12:0) were obtained from Sigma Chemical Company. Lipase TLIM was a gift from Novozymes India Pvt. Ltd. All other reagents used were of analytical grade and procured from Merck India Ltd., Mumbai, India.

Preparation of Oils:

The reaction between caprylic acid, capric acid and lauric acid with rice bran oil was carried out in a packed-bed bioreactor. The reactor consisted of a tubular glass column of 10 mm ID and was 50 cm long. It was also provided with a water jacket for temperature control. The immobilised enzyme (*Rhizomucor mehei*) packed into the reactor was retained in place by means of a sintered plate. The substrates were fed from the top and the products were collected at the bottom. The substrates were previously blended and well mixed at the reaction temperature before conducting the packed-bed reaction and were poured into the enzyme bed, maintaining a fixed sample head. Water from a constant temperature bath was circulated through the jacket by a peristaltic pump. A partial suction was given to maintain the constant flow rate (0.4 mL/min; optimized in the previous study); 20 g of enzyme was closely packed into the column by repeated tapping to avoid any air gaps. Transesterification reactions were then carried out by passing the substrate through the column. The temperature was maintained at the desired value of 60ºC by passing water through the column jacket. The product mixture was collected at the outlet, steam stripped for removal of excess fatty acids and the fatty acid composition of the oils was determined by gas chromatography (GC).

Chromatographic analysis of oils:

Fatty acid compositions of native and MCFA rich rice bran oil were analysed by GC. The oils were saponified with 0·5 M KOH and methylated with boron trifluoride in methanol. The gas

chromatograph (Agilent 6890 N; J&W Scientific, Wilmington, DE, USA) was fitted with a DB-Wax capillary column (30m x 0·32mm x 0·25 mm) and a flame ionization detector. N_2, H_2 and airflow rate were maintained at 1, 30 and 300 ml/min, respectively. Inlet and detector temperatures were kept at 250ºC and the oven temperature was programmed to increase from 150 to 190°C at a rate of 15°C/min, then to hold for 5 min, and then to increase to 230°C at a rate of 48°C/min, and then again to hold for 10 min.

Physicochemical Property Analysis:

Saponification value (SV)

It was determined using AOCS method (1990a). 2.0g oil samples were added with ethanolic potassium hydroxide 0.5N and boiled for 60 min in a reflux condenser. The mixtures were cooled and subsequently titrated with 0.5N hydrochloric acid until the colour of the mixtures changed from pink to the original colour.

Iodine value (IV)

The determination of IV was carried according to AOCS method (1990b). Samples were reacted with the Wij's solution and left in the dark for 1 h. Mixture was consequently titrated with sodium thiosulphate solution.

Viscosity

The viscosity of the formulations was determined using Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) using spindle # CPE40 at 25 ± 0.5 °C. The software used for the calculations was Rheocalc V2.6 (Akoh and Min, 1998).

Refractive Index

Refractive index was determined using an Abbes type refractometer (Nirmal International, New Delhi, India) at 25 ± 0.5 °C (Chong *et al*., 1971).

Slip Melting Point

Slip melting point was determined using slip melting apparatus by capillary tube method (A.O.C.S. 1990c).

In vitro intestinal digestion study:

The in vitro gastric digestion protocol simulated fasted-state conditions in humans with a pH between 1 and 3[16]. First, 10 mL of simulated gastric fluid (SGF) (per liter, 2 g of NaCl and 7 mL of HCl at pH 1.226) was mixed with 20 mL of milk, which was then acidified to pH 1.5 with 6 M HCl and incubated at 37°C for 10 min in a shaking water bath at 95 revolutions/min. Then, pepsin was added at the physiologically relevant substrate/enzyme ratio (w/w) of 20:1, and the temperature and pH were kept constant for 1 h. Samples were collected periodically for further characterization.

The simulated intestinal fluid (SIF) (per liter, 6.8 g of K_2HPO_4 and 190 mL of 0.2 M NaOH at pH 7.527) contained 150 mM NaCl to simulate the in vivo intestinal ionic strength and bile extract (5mg/mL). No calcium was added because bile extract may already contain physiological concentrations of calcium (Kalantzi *et al*., 2006). Experimental oils were subjected to gastric digestion for 1 h, as described above. Then, thein vitro intestinal digestion carried out by mixing the experimental oils with SIF (1:3, v/v) to a total of 30 mL in a conical flask. The pH was adjusted to 7, and the mixture was placed in a shaking water bath (95 revolutions/min) at 37 °C. Pancreatin (1.6 mg/mL) was added to the mixture; the pH was maintained at 7 with 1 M NaOH; and samples were taken periodically over 2 h for characterization. The activity of pancreatic lipase was measured over 2 h using a pH stat titration method (TitraLab 856, Radiometer Analytical, Villeurbanne, France) with 0.05 M NaOH and an end point of pH 7.0. The total free fatty acids released were back-titrated at different time points of intestinal digestion, as described in literature (Tiss *et al*., 2001).

In vivo absorption study rats:

In the present study, we have used the single pass perfusion method in rat model (Gallier and Singh, 2012). Experiments were carried out under the supervision of Animal Ethical Committee of the department of Chemical Technology, University of Calcutta. Male Sprague-Dawley rats (150-250 g) were acclimatized for a week in laboratory condition, gave diet food and water ad libidum. Each animals were fasted overnight and then anesthetized with intra-muscular injectrion of

Ketajet 50 @ 35mg/kg body weight and intra-peritoneal injection of Xylocaine 2% @ 5mg/kg body weight. The peritoneal cavity was then opened by a midline incision. The small intestine was cannulated proximally (just downstream to the pyloric sphincter) and distally (about 5mm upstream to the illeo-caecal junction) so that the perfusate (emulsion in this case) entering the small intestine, traversed through it and finally left through the distal cannula, provided with a stoppered cap. The cannula was tied in place with a loop of silk suture placed tightly about the intestine at its both end. A similar tie-up has been given on gastric end of oesophagus to prevent any accidental backflow of the perfusate though the nasopharyngeal channel and subsequent choking of the respiratory system.

The cannulated intestine is arranged in the peritoneal cavity so that it was not kinked or twisted. The cannulated intestine is kept covered throughout the experimental time-period with a cotton cloth soaked in phosphate buffer saline (PBS) to prevent drying. Firstly the intestine is flushed with the perfusate until the outflow fluid became clear and clean. Now a fixed volume (6ml) of the experimental oils is flown into the cannulated small intestine and after a stipulated time period of residence there (different time intervals in between 0 and 30 min) the outflow-fluid is collected for subsequent analysis. The difference of volume between the perfusate-oil and the outflow-oil was taken as the 'absorbed volume'.

Total lipid content from the experimental from the experimental oils was determined using standard method (Tarr and Yalkowsky, 1989). The difference between the lipid content of initial oil and outflow oil was taken as 'absorbed lipids'.

Statistical Analysis:

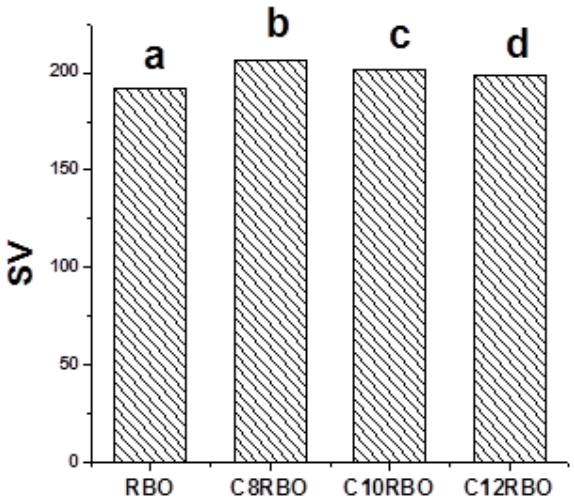
All the data are presented as means with their standard errors. Statistical comparisons between groups were performed using one way ANOVA.

RESULTS AND DISCUSSION

Changes in fatty acid composition:

Analysis of the MCFA rich RBO showed that caprylic acid rich RBO contained 14.00% caprylic acid (C8), capric acid rich RBO contained 13.43% capric acid (C10) and lauric acid rich RBO contained 13.11% lauric acid (C12). The fatty acid compositions of the MCFA rich RBOs, are given in Figure 1.

Figure 1. Changes in saponification value of the oil samples. (RBO-Rice bran oil; C8RBO-caprylic acid rich rice bran oil; C10RBO-capric acid rich rice bran oil; C12RBO-lauric acid rich rice bran oil) Values are Mean±S.D Values not sharing common superscript are significantly different ($p<0.05$)



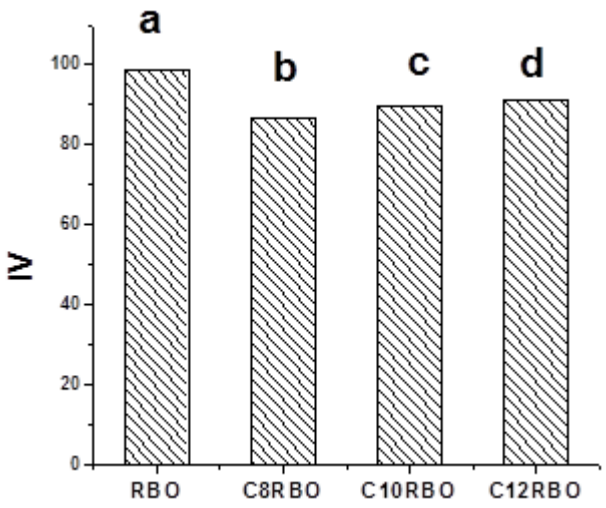
Changes in saponification value (SV):

The SV of all experimental oils showed values ranging from 191-206. The highest SV was from and the lowest was recorded in RBO (Fig 1). The SV basically refers to the mean molecular mass of the fats and oils and have an inverse relationship with the chain length of the fatty acid present in the fats and oil. This means, longer the average fatty acid chain length, the smaller the SV. Thus in this case as the chain length of fatty acids present in RBO was long it had lowest SV. Similarly since caprylic acid rich RBO had the lowest chain length, it had the highest SV.

Changes in Iodine Value:

The iodine value performed on the experimental oils is shown in Fig 2. The range of IV of all samples was between 86-99. The lowest IV was obtained in case of MCFA rich RBO and highest in case of RBO.

Figure 2. Changes in iodine value of the oil samples. (RBO-Rice bran oil; C8RBO-caprylic acid rich rice bran oil; C10RBO-capric acid rich rice bran oil; C12RBO-lauric acid rich rice bran oil) Values are Mean±S.D Values not sharing common superscript are significantly different (p<0.05).



The low IV in case of medium chain fatty acid rich RBO was low owing to the high degree of saturation. This highlights the low likeliness of MCFA rich RBO to become rancid from lipid oxidation (Folch *et al.* 1951) . The IV would have effects on the overall quality of the MCFA rich RBO such as shelf life of the oils, appearance as well as taste and smell.

Changes in viscosity:

The viscosity of RBO is shown to portray lowest viscosity as depicted in Fig 3. In comparison to RBO MCFA rich RBO showed higher viscosity. Among the three MCFA rich RBOs lauric acid rich RBO showed the highest viscosity and caprylic acid rich RBO showed the lowest viscosity.

Changes in slip melting point:

From Fig 4 it was evident that RBO had highest slip melting point in comparison to MCFA rich RBO. This was due to the higher unsaturation of RBO than MCFA rich RBO. Among the three MCFA rich RBOs lauric acid rich RBO had the highest slip melting point due to the longer chain length of lauric acid in comparison to caprylic acid.

Figure 3. Changes in viscosity in oil samples. (RBO-Rice bran oil; C8RBO-caprylic acid rich rice bran oil; C10RBO-capric acid rich rice bran oil; C12RBO-lauric acid rich rice bran oil) Values are Mean±S.D Values not sharing common superscript are significantly different (p<0.05).

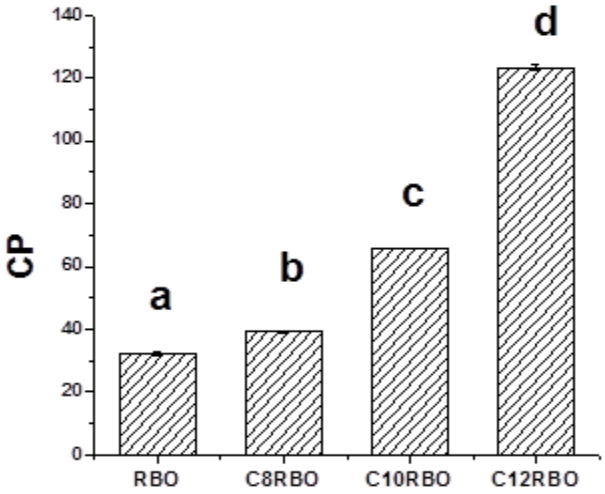
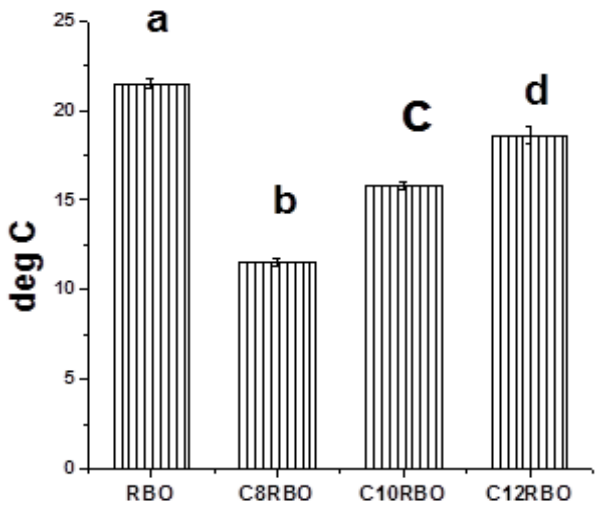


Figure 4. Changes in slip melting point in oil samples. (RBO-Rice bran oil; C8RBO-caprylic acid rich rice bran oil; C10RBO-capric acid rich rice bran oil; C12RBO-lauric acid rich rice bran oil) Values are Mean±S.D Values not sharing common superscript are significantly different (p<0.05).



Changes in refractive index:

Refractive index is a very important physical property of fats and fatty acids as it is a unique method for their identification. Refractive index of fats and fatty acids depends on the chain length and unsaturation. With increase in chain length and double bonds, refractive index increases. RBO showed the highest refractive index and caprylic acid rich RBO the lowest.

Figure 5. Total free fatty acid release from gastric digested oil samples during 120 min of intestinal digestion and measured after back titration. (RBO-Rice bran oil; C8RBO-caprylic acid rich rice bran oil; C10RBO-capric acid rich rice bran oil; C12RBO-lauric acid rich rice bran oil).

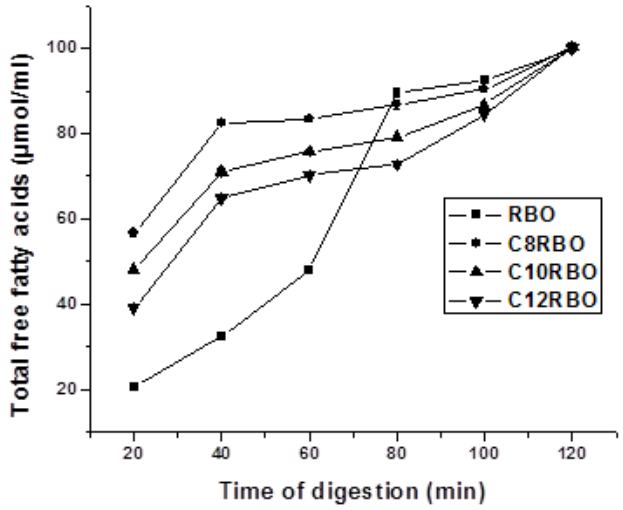
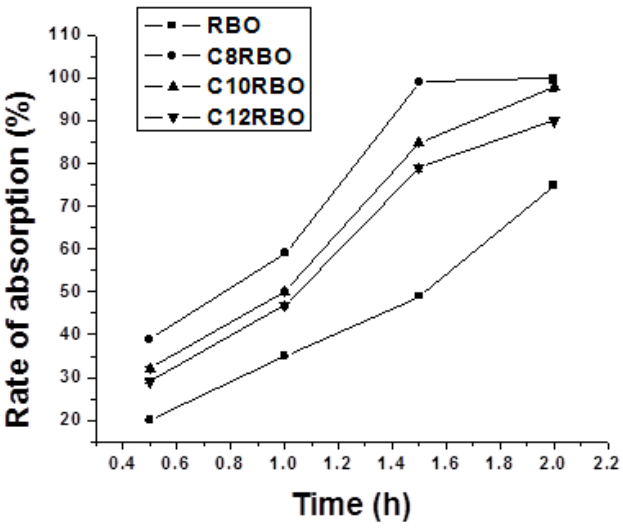


Figure 6. Rate of absorption of oil samples during 2 hr of intestinal absorption. (RBO-Rice bran oil; C8RBO-caprylic acid rich rice bran oil; C10RBO-capric acid rich rice bran oil; C12RBO-lauric acid rich rice bran oil).



Free fatty acid release after in vitro intestinal digestion:

The release of total free fatty acids is depicted in Fig 5. From the curve it was evident that the rate of lipolysis was fastest in caprylic acid rich RBO and slowest in case of RBO. This was due to the presence of higher amounts of long chain fatty acids in RBO in comparison to MCFA rich RBO. The rate of lipolysis was high in MCFA rich RBO for the first 30 min and then slowed down. On the other hand the rate of lipolysis in case of RBO was gradually increasing till 90 min and then slowed down. The lipolytic products are further solubilized into phospholipid vesicles and mixed phospholipid-bile salt micelles (Onyeike and Acheru, 2002). No lag phase was observed in the titration curve of the release of titrable free fatty acids, suggesting that pancreatic lipase was rapidly adsorbed and activated at the surface of experimental oils. The rate of lipolysis slowed down progressively after sometime indicating that the free fatty acids were almost released by that time. MCFA rich RBOs were mostly lipolized by gastric lipase.

Changes in in vivo intestinal lipid absorption in rats:

The results of in vivo absorption study based on intestinal perfusion model are depicted in Fig 6. RBO and all three MCFA rich RBOs were passed through the intestinal perfusion model and the gastrointestinal absorption was measured thereby. The absorption study was conducted for 2 h. The sample was collected at every half an hour interval. The rate of absorption of MCFA rich RBOs was much faster than native RBO. Caprylic acid rich RBO was absorbed at a fastest rate.

Fatty acid chain length affects aqueous solubility and also appears to have an important influence on the molecular mechanisms that govern uptake of these molecules. Importantly, MCFAs with 6–12 carbon atoms, have increased water solubility, and this means that they have measurable absorption via the paracellular route. A consequence of this is that such MCFAs appear to bypass the intracellular processing events that are encountered by the long chain fatty acids that predominantly enter the enterocyte cytosol. As a result, such fatty acids also follow a different route out of the gut, being exported chiefly via the portal circulation rather than the lymphatic route used by other lipids. The long chain fats, by contrast, are quite insoluble in water, the release of bile salts are required to emulsify these fats, allowing pancreatic lipase enzymes to start to break them down. The resulting micelles can diffuse across the brush border membrane of the gut and into the enterocytes. Once there, the long-chain fatty acids

are re-esterified back into their triglyceride form, pumped toward the lymph system, the thoracic duct, and eventually the bloodstream via the water-soluble lipoproteins.

CONCLUSION

RBO is an unique oil which is well known for its oryzanol content which renders it high antioxidative property. Enzymatic conversion of RBO to MCFA rich RBO decreases the peroxide value and increases the para-ansidine value. Modification of RBO to MCFA rich RBO increases the digestion and absorption property of RBO due to the replacement of long chain fatty acid with medium chain fatty acid. Thus better absorption of RBO offers better availability of RBO to provide its antioxidative property. Among the three MCFA rich RBO, caprylic acid rich RBO has better ameliorative property.

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