

In vitro antioxidant assay of medium chain fatty acid rich rice bran oil in comparison to native rice bran oil

Avery Sengupta · Mahua Ghosh · D. K. Bhattacharyya

Revised: 18 July 2014 / Accepted: 26 August 2014 / Published online: 23 September 2014
© Association of Food Scientists & Technologists (India) 2014

Abstract The study aimed to evaluate the in vitro antioxidant activity of medium chain fatty acid (MCFA) rich-rice bran oils in comparison with native rice bran oil. Different in vitro methods were used to evaluate the free radical scavenging activity, metal chelation activity, reducing activity, ABTS radical scavenging activity, thiobarbituric acid (TBA) value and so on at different concentrations of the oils such as 10–100 µg/mL. Inhibition of lipid peroxidation was evaluated measuring thiobarbituric acid responsive substance (TBARS) and conjugated diene formation. All the oils showed potent antioxidant activity at 100 µg/mL concentration. TBARS formation and conjugated diene formation was lower with MCFA rich oils i.e. the inhibition of lipid peroxidation was more in MCFA rich oils than original rice bran oil. Caprylic acid rich rice bran oil showed maximum antioxidant activity in comparison to capric- and lauric acid rich rice bran oils. Overall the MCFA rich rice bran oils showed to be more potent antioxidant than rice bran oil due to their lower unsaturated fatty acid content.

Keywords Rice bran oil · Medium chain fatty acids · In vitro antioxidant · Saturated fatty acids

Introduction

Oils and fats are important part of human diet. They are rich source of energy containing fatty acids, antioxidants, anti-foaming and anti-surfactant. Vegetable oils play important

functional and sensory roles in food products and they act as carriers of fat soluble vitamins. Lipids are better protected against oxidation by addition of antioxidants that remove the free radicals and reactive oxygen species.

Rice bran (RBO) is widely used as a cooking oil in the Asian countries and it is gaining acceptance in western countries also. It is considered to be relatively stable vegetable oil. RBO is considered useful for health and cooking because of its high level of unsaponifiable matter comprised of gamma-oryzanol, tocotrienols and phytosterols, which are bioactive components possessing powerful antioxidative activity (Cheruvanky 2000). The bioactive components present help in preventing the damage of body tissue and oxidative damage of DNA. These components are used in the development of value-added healthy products (Wells 1993). Due to the antioxidant action, RBO is drawing immense interest in the world of food additive where it is termed as ‘oxidation inhibitor’. RBO also has hypocholesterolemic influence resulting from selective decrease of LDL-Cholesterol fraction (Lichtenstein et al. 1994). RBO has shown to display immunostimulation effects (Sierra et al. 2005).

Medium chain fatty acids (MCFA) are fatty acids with carbon number 8–12. MCFA containing oils are special-purpose food for use as supportive nutritional therapy. It may be used to decrease the calorie value, improve the palatability, digestibility and absorption. It has a number of properties that may be beneficial in preventing atherosclerosis; among these are that MCFAs have anti-coagulating effects, and have been shown to lower plasma cholesterol. In addition, MCFAs reduce levels of cholesterol in the liver and other tissues. In one of the recent publications it has been suggested that natural edible oils at present, consumed primarily as cooking oil should be modified by introducing the MCFAs displacing the long chain acids particularly the saturated acids for the purpose of imparting their nutritional quality and health benefits.

The debate over the beneficial effects of saturated and unsaturated fatty acids is still under research. Unsaturated fatty acids are beneficial for the prevention of coronary heart

A. Sengupta (✉) · D. K. Bhattacharyya
School of Community Science and Technology, Indian Institute of Engineering, Science and Technol., Shibpur (BESUS),
Howrah 711103, India
e-mail: avery.sgupta@gmail.com

M. Ghosh
Department of Chemical Technology, University of Calcutta,
Kolkata, India

disease and reduce platelet aggregation. On the other hand saturated fatty acids undergo less lipid peroxidation than unsaturated fatty acids. Lipid peroxidation is harmful to human body in causing a cascade of life-threatening diseases. Thus antioxidant comes into play to reduce the lipid peroxidation in the body. RBO acts as a good source of antioxidant due to the presence of the bioactive compounds but it is rich in unsaturated fatty acids. Thus the study aims in production of a structured lipid enzymatically by introduction of medium chain fatty acids into RBO in order to examine whether the antioxidant property of RBO enhances even further. In vitro antioxidant assay was performed to compare the effect of rice bran oil with that of the medium chain fatty acid-rich rice bran oil from the antioxidant point of view.

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 at 3:1 molar ratio of fatty acid:oil for 6 h. 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 60 °C by passing water through the column jacket. The product mixture was collected at the outlet and the product was steam stripped to remove the excess fatty acid from the reaction product. The fatty acid composition of the oils was determined by gas chromatography (GC).

Chromatographic analysis of oils

Fatty acid compositions of native and medium chain fatty acid-enriched 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 (30 m×0.32 mm×0.25 mm) and a flame ionization detector. N₂, H₂ and airflow rate were maintained at 1, 30 and 300 ml/min, respectively. Inlet and detector temperatures were kept at 250 °C 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.

Preparation of oil samples

Oil samples were prepared after dissolving them in DMSO (dimethyl sulfoxide). Preparation of oil samples for assay of different antioxidative enzymes were shown in Table 1.

Antioxidant assays

DPPH assay

The free radical scavenging activity of rice bran oil and medium chain fatty acid rich rice bran oils dissolved in DMSO was measured by DPPH. Various concentrations of the oil were made upto 1 mL using adequate amount of DMSO; 1 mL of DPPH solution (0.2 mM) was added and kept for incubation at room temperature in dark for about 30 min. A control and blank was also performed simultaneously. The absorbance was read at 517 nm using spectrophotometer. Gallic acid was used as a standard. The percentage radical scavenging capacity was determined using the following formula:

$$\% \text{RSC} = [(A_o - A_s) / A_o] \times 100$$

Where A_o is the absorbance of control, and A_s is the absorbance of tested samples.

Estimation of reducing activity

Reducing activity was measured according to the method of Oyaizu (Oyaizu 1986). Increase in antioxidant activity was indicated by increase in absorbance. Different concentrations of the samples and methanol were taken in a centrifuge tube. 2.5 mL of 1 % potassium ferricyanide [$K_3Fe(CN)_6$] was added. Total preparation was incubated at 50 °C for 20 min. After cooling 2.5 mL of 10 % TCA was added to it and the

Table 1 Preparation of oil samples for antioxidant assays

Sample	Concentration (µg/mL)	DMSO (mL)
Rice Bran Oil and MCFA rich Rice Bran Oil	10	1
	20	1
	30	1
	50	1
	100	1

mixture was centrifuged at 3,000 rpm for 10 min, then 2.5 mL fraction of supernatant was mixed with 2.5 mL of 0.1 % FeCl₃ and left to stand for 10 min. Absorbance was measured at 700 nm. All analysis were run in triplicate and averaged.

Estimation of metal chelation

Metal chelation activity was determined according to the method reported in the studies of Shyamala et al. (Shyamala et al. 2005). Different concentration of samples were taken in different test tubes and mixed with 3 mL of methanol. 50 mL of 2 mmol/L FeCl₂ was added to it. 0.2 mL of 5 mmol/L Ferrozine was added and centrifuged at 5,000 rpm for 5 min. Absorbance of supernatant was taken at 562 nm.

Estimation of hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured according to the protocol previously reported (Singh et al. 2002). Different concentrations of samples were taken in different test tubes. 1 mL of Fe-Ethylenediaminetetraacetic acid (Fe-EDTA) solution (0.1 % ferrous ammonium sulphate and 0.26 % EDTA), 0.5 mL EDTA (0.018 %), 1 mL of DMSO (0.85 % v/v in 0.1 mol/L phosphate buffer, pH 7.4) were added to these tubes. The reaction was initiated by adding 0.5 mL of 0.22 % ascorbic acid. Test tubes were capped tightly and heated on a water bath at 90 °C for 15 min. The reaction was terminated by the addition of 1 mL of cold trichloroacetic acid (TCA) (17.5 %). 3 mL of Nash reagent (75 g of ammonium acetate, 3 mL of glacial acetic acid and 2 mL of acetyl acetone were mixed and raised to 1 L with methanol) was added to all of the test tubes and left at room temperature for 15 min for colour development. The mixture was centrifuged at 5,000 rpm for 5 min. The intensity of the yellow colour formed was measured spectrophotometrically at 412 nm against blank. The percentage of hydroxyl radical scavenging activity is calculated as follows:

% Hydroxyl Radical Scavenging Activity

$$= (1 - \text{Absorbance of Sample} / \text{Absorbance of Blank}) \times 100$$

Inhibition of lipid peroxidation

Estimation of TBARS by FTC Method

The ferric thiocyanate method (Ozsoy et al. 2008) was used to determine the inhibition of lipid peroxidation. A linoleic acid emulsion (0.02 mol/L) was prepared with linoleic acid (0.28 mg) and Tween 20 (0.28 mg) in phosphate buffer at pH 7.4 (50 mL, 0.05 mol/L). Different concentrations of samples were taken. 2.5 mL of linoleic acid emulsion and 2.3 mL of phosphate buffer (0.2 mol/L, pH 7.0) were added to the sample and was homogenized on ice. The reaction mixture was incubated at 37 °C in the dark for an hour. To 0.1 mL of the reaction mixture were added 4.7 mL of 75 % ethanol and 0.1 mL of 30 % ammonium thiocyanate. After 3 min, 0.1 mL of 0.02 mol/L FeCl₂ in 3.5 % HCl was added, and the absorbance of the supernatant was measured at 534 nm in a spectrophotometer (Shimadzu, Tokyo, Japan) every 24 h for 4 days, and the relative amounts of lipid peroxides were expressed in absorbance units, A534 nm.

Estimation of conjugated diene formation

In this method the progress of autoxidation was monitored by UV absorbance at 234 nm (Amax of conjugated diene peroxides from linoleic acid oxidation) (Kleinveld et al. 1992). After incubation mixture was shaken and 1 mL taken in a separating funnel. 5 mL of chloroform:methanol (2:1) was added to it and shaken and left to stand for 15–30 min., for layer separation. After separation of linoleic acid and experimental oil in a separating funnel, 0.2 mL of lower organic layer was taken in a centrifuge tube by passing through sodium sulphate. 3 mL of iso-octane was added and absorbance was measured at 233 nm. Diene concentration was measured by the following formula using absorbance of the sample:

$$\% \text{ Conjugation of dienes } 0 : 84 \times (\text{Absorbance}/B \times C) - K0$$

K0 0.03

B cell length

C concentration of linolenic acid in g/L

Thiobarbituric acid value (TBA)

The TBA value of different samples was determined using the method of Marcuse et al. (Marcuse, & Johansson, 1973). About 100 mg of oil sample was dissolved in 25 mL of 1-butanol. A 25 mL aliquot of the above solution was mixed thoroughly with 5.0 mL of TBA reagent (200 mg of TBA in 100 mL of 1-butanol) and heated at 90 °C for 10 mins. The absorbance was measured at 530 nm. At the same time reagent

blank was also done. The thiobarbituric acid value (mequiv of malonaldehyde/g) was calculated as

$$\text{TBA value} = \frac{50 \times (A-B)}{M}$$

where A=absorbance of test sample, B=absorbance of blank sample and M=mass of the sample (mg)

ABTS radical scavenging activity

The experiment was carried out using an improved ABTS decolorization assay, which involves the generation of ABTS+chromophore by the oxidation of ABTS with potassium persulfate. It is applicable for both hydrophilic and lipophilic compounds. The ABTS radical cation was generated by adding 7 mM stock solution of ABTS and 2.45 mM potassium persulfate together in 10 mL distilled water and was allowed to stand overnight in the dark at room temperature. The absorbance was maintained to 0.65–0.75 range with ethanol before the assay (Arnao et al. 2001). Several concentrations of the samples were prepared and added to 800 mL ABTS solution. The solutions were allowed to stand for 8 min. A control and blank was also performed simultaneously. Experiments were carried out in triplicate, and the mean values were taken. The absorbance was read at 734 nm at kinetic mode.

Nitric oxide radical scavenging assay

The nitric oxide scavenging ability of the extracts was observed by its reaction with sodium nitroprusside (SNP) (Marcocci et al. 1994). The compound SNP is known to decompose in aqueous solution at physiological pH (7.2), producing nitric oxide. Under aerobic conditions, nitric oxide reacts with oxygen to produce stable products (nitrate and nitrite), the quantities of which can be determined using Griess reagent. Various concentrations of the samples were taken and made up to 100 mL using phosphate-buffered saline at pH 7.44. To this, 1 mL of SNP solution (5 mM) was added. The tubes were incubated at 29 °C for 150 min. An aliquot (100 mL) of the incubated solution was removed and diluted with 100 mL of Griess reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediaminedihydrochloride was immediately read at 546 nm, and a graph was plotted with concentration along X-axis and absorbance along Y-axis. TBHQ was used as the standard. The percentage radical scavenging capacity was determined using the following formula:

$$\% \text{ RSA} = \left[\frac{(A_o - A_s)}{A_o} \right] \times 100$$

where A_o is the absorbance of control without tested samples, and A_s is the absorbance of tested samples, and IC50 values were calculated and expressed in $\mu\text{g/mL}$.

β -Carotene bleaching

Antioxidant activity of essential oils was determined using β -Carotene bleaching test (Abd El-Baky and El-Baroty 2008). Approximately 10 mg of β -Carotene was dissolved in 10 ml of chloroform. The carotene-chloroform solution, 0.2 ml, was pipette into a boiling flask containing 20 mg linoleic acid and 200 mg Tween 40. Chloroform was removed using a rotary evaporator to the residue, 50 ml of distilled water was added slowly with rigorous agitation to form an emulsion. Five ml of the emulsion were added to a tube containing 0.2 ml of oil samples dissolved in DMSO and the absorbance was measured immediately measured at 470 nm against blank, consisting of an emulsion without the carotene. The tubes were placed in water bath at 50 °C and the oxidation was measured spectrometrically by measuring the absorbance at 470 nm over a period of 60 min. Control samples contained 10 μl of water instead of the oil samples. The antioxidant activity was expressed as inhibition percentage with reference to the control after a 60 min incubation using the following equation: $AA = 100(DR_c - DR_s)/DR_c$, where AA=antioxidant activity; DR_c =degradation rate of the control= $[\ln(a/b)/60]$; DR_s =degradation rate in presence of the sample= $[\ln(a/b)/60]$; a=absorbance at time 0; b=absorbance at time 60 min.

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 medium chain fatty acid 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 Table 2.

Scavenging effect on DPPH

RBO and MCFA rich RBO showed a dependent DPPH radical scavenging effect. From Fig. 1 it is evident that as the concentration of sample increased, the scavenging effect also

Table 2 Fatty acid composition of native rice bran oil and medium chain fatty acid rich rice bran oils

Fatty acid → ↓ Sample	Fatty acid (% w/w)								
	C _{8:0}	C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}
Rice Bran Oil	–	–	–	0.28±0.03	17.10±0.09	1.90±0.06	48.27±0.12	31.35±0.55	1.1±0.02
Caprylic acid rich RBO	14.00±0.14	–	–	0.29±0.03	13.77±0.11	1.90±0.03	40.76±0.16	28.28±0.20	1.0±0.01
Capric acid rich RBO	–	13.43±0.32	–	0.27±0.02	10.44±0.10	1.89±0.02	43.39±0.28	29.58±0.29	1.0±0.01
Lauric acid rich RBO	–	–	13.11±0.12	0.27±0.02	10.56±0.18	1.88±0.01	45.29±0.22	27.89±0.21	1.0±0.01

Values are Mean±S.D (n=3)

increased. MCFA rich RBO showed better scavenging effect than RBO among which caprylic acid rich RBO showed maximum scavenging effect.

Changes in reducing power

For the measurement of reducing ability of Fe³⁺ to Fe²⁺ transformations in the presence of oil samples are shown in Fig. 2. From the figure the reducing power of all samples was found to be significantly higher in MCFA rich rice bran oil than in RBO and depended on the concentration of the oil samples. Moreover the reducing power of caprylic acid rich RBO was much higher in comparison to lauric acid rich RBO.

Changes in metal chelation activity

The metal chelation activity of the oil samples are shown in Fig. 3. From the figure the metal chelation activity of MCFA rich rice bran oil was found to be significantly higher than RBO and The metal chelation activity increased with the increase in the concentration of the oil samples. Moreover

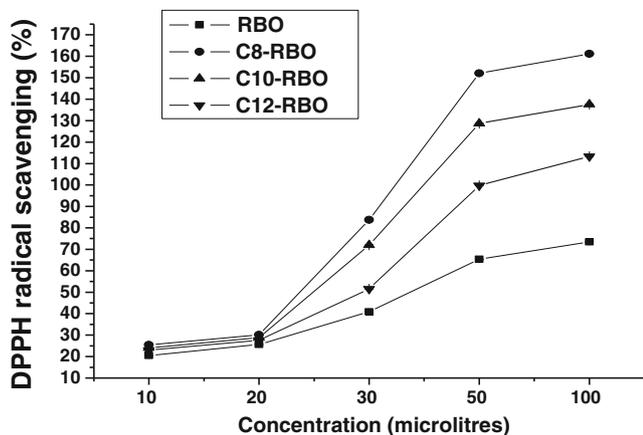


Fig. 1 Radical scavenging effect of oil samples on 2,2-diphenyl-1-picrylhydrazyl radical (Values are Mean±S.D)

the metal chelation activity of caprylic acid rich RBO was much higher in comparison to lauric acid rich RBO.

Changes in hydroxyl radical scavenging activity

This method was based on scavenging of the hydroxyl radicals in presence of antioxidant. The Fig. 4 shows the changes in hydroxyl radical scavenging activity of oil samples. Results depicted that all the oil samples possess a hydroxyl radical scavenging activity, but there was a significant difference in the scavenging activity of RBO and MCFA rich RBO. MCFA rich RBOs showed much better scavenging activity in comparison to RBO.

Changes in TBARS by FTC method

The initial value of lipid peroxidation was measured using a linoleic acid system. In this assay, a hydroperoxide formed from a lipid i.e. linoleic acid oxidizes a ferrous ion to a ferric ion which is monitored by a thiocyanate complex spectrophotometrically. The inhibitory effect toward oxidation from ferrous ion to ferric ion by antioxidants is evaluated by

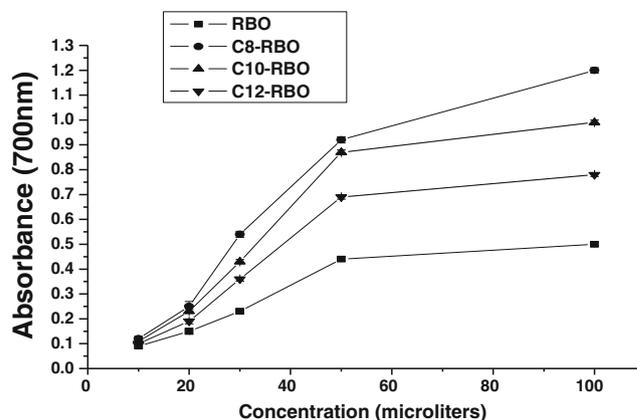


Fig. 2 Antioxidative effect of oil samples in reducing power system (Values are Mean±S.D)

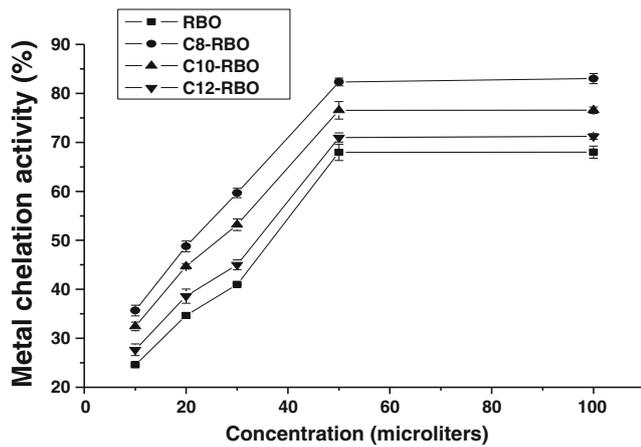


Fig. 3 Antioxidative effect of oil samples in metal chelation activity (Values are Mean±S.D)

monitoring the formation of ferric thiocyanate complex. A low absorbance value indicates a high level of antioxidant activity. From the inhibitor activities against lipid peroxidation in linoleic acid caused by oil samples shown in Fig. 5 it is clear that MCFA rich RBOs have higher antioxidant activity than RBO. Caprylic acid rich RBO showed the highest antioxidant activity. The activity increased with the increase in the number of days of incubation.

Changes in conjugated diene formation

The term conjugated diene is defined as a moiety with two double bonds separated by a single bond. This kind of moiety doesnot normally occurs in unsaturated fatty acids. However, a conjugated diene is readily formed from a moiety with two double bonds separated by a single methylene group, which occurs most commonly in polyunsaturated fatty acids, by the action od reactive oxygen species and oxygen. Once conjugated diene is formed it can be monitored spectrophotometrically

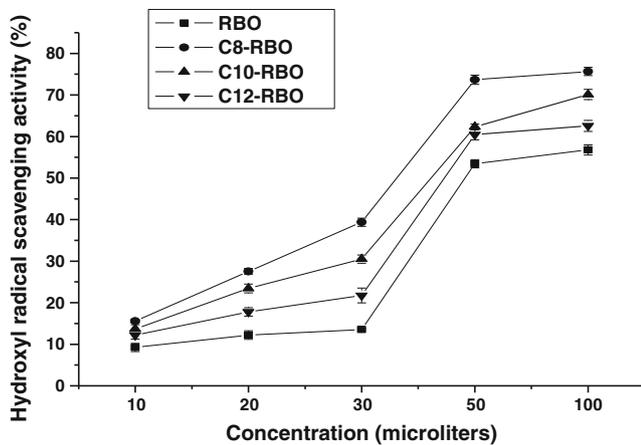


Fig. 4 Hydroxyl radical scavenging activity of oil samples (Values are Mean±S.D)

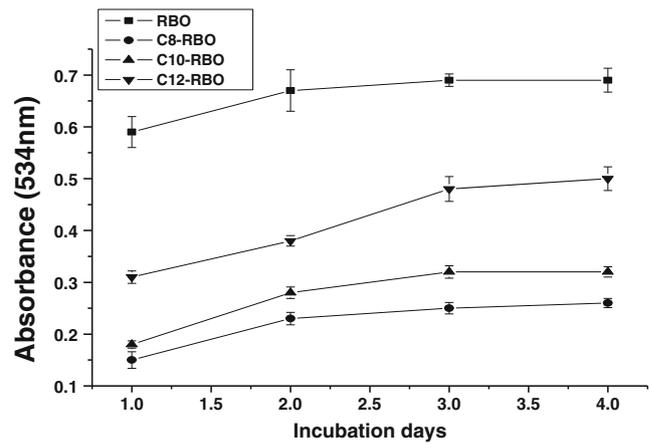


Fig. 5 Inhibition of lipid peroxidation by oil samples (Values are Mean±S.D)

(Moore and Roberts 1998). Conjugated diene formation assay was performed with 100 µl of oil sample as this concentration showed highest antioxidant activity. Results from Fig. 6 showed the higher inhibitory effect of MCFA rich RBO in comparison to RBO on diene formation during lipid peroxidation. Number of days increased the diene formation by incorporation of MCFA in RBO.

Changes in TBA value

During the oxidation process, peroxides are generally decomposed to lower molecular weight compounds; one such compound is malondialdehyde, which is measured by TBA method. Malondialdehyde is used as an index of lipid peroxidation which was determined by a third order derivative spectrophotometric method. From Fig. 7 it is evident that MCFA rich RBO is more effective in comparison to RBO. Moreover caprylic acid rich RBO is more effective against the oxidation process.

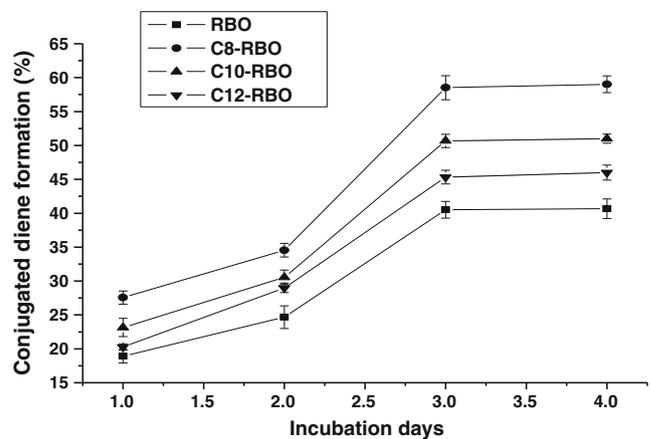


Fig. 6 Inhibition effect of oil samples on conjugated diene formation during lipid peroxidation (Values are Mean±S.D)

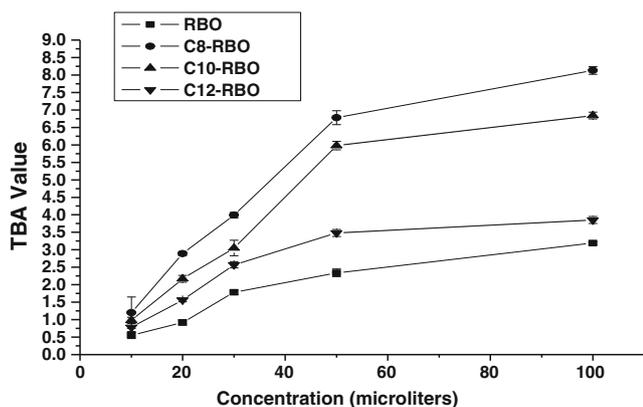


Fig. 7 Antioxidative effect of oil samples in terms of TBA Value (Values are Mean±S.D)

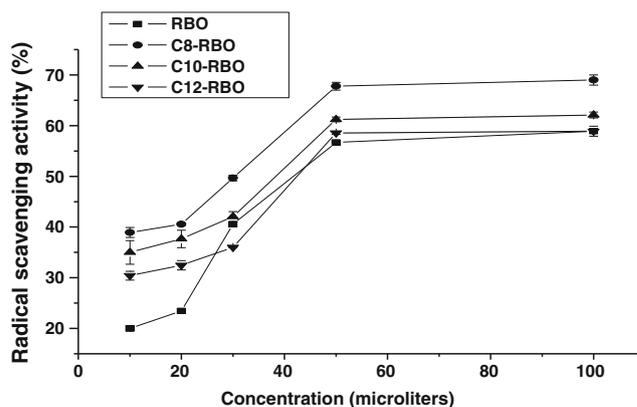


Fig. 9 Nitric oxide radical scavenging activity of oil samples (Values are Mean±S.D)

Changes in ABTS radical scavenging activity

The ABTS assay, which is also called the ABTS radical assay, has been widely used to evaluate antioxidant activities of different food components due to its applicability in both aqueous and lipid phases (MacDonald-Wicks et al. 2006). A stable ABTS radical cation, which has a blue-green chromophore absorption, was produced by oxidation of ABTS with potassium persulphate prior to addition of antioxidants (Re et al. 1999). The antioxidant capacity of the oil samples are determined by the decolourization of the ABTS, by measuring the reduction of the radical cation as a percentage inhibition of absorbance. The oil samples were able to reduce the stable radical, indicating that the oil possessed hydrogen-donating capabilities and acted as antioxidants, which can scavenge the long-lived ABTS radical cation. Results of the ABTS radical scavenging assay as depicted in Fig. 8 shows that caprylic acid rich RBO showed maximum radical scavenging activity and RBO showed the least. With the increase in concentration of oil samples the ABTS radical scavenging increased.

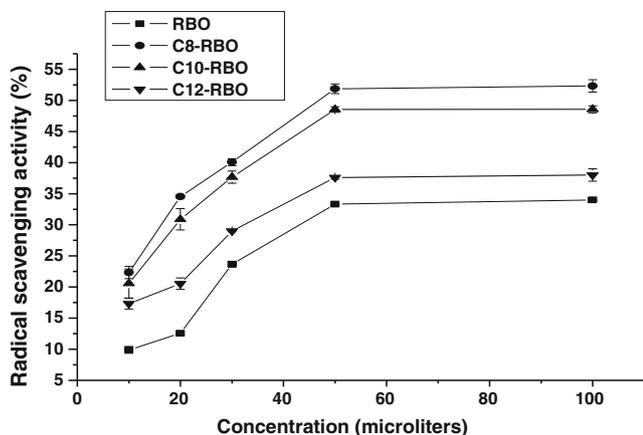


Fig. 8 ABTS radical scavenging activity of oil samples (Values are Mean±S.D)

Changes in Nitric oxide radical scavenging activity

Nitric oxide radicals produced by vascular endothelium is important in the regulation of blood flow, but at different pathological conditions, excess nitric oxide radicals are formed, and this in turn affects the blood flow. The excess nitric oxide formed in the body must be scavenged to avoid their harmful ill effects to cellular components that results in inflammation, cancer and severe other disease conditions (Alderton et al. 2001). Caprylic acid rich RBO showed the highest nitric oxide radical scavenging activity and RBO alone showed the least (Fig. 9).

Changes in β-Carotene bleaching

It has long been known that β-Carotene reacts with the peroxy radical to produce β-Carotene epoxides (Tsuchihashi et al. 1995). Therefore, β-Carotene has received attention as a radical scavenger or antioxidant. Later an antioxidant assay using β-Carotene combined with lipids, such as linoleic acid, was established. Linoleic acid form a peroxy radical in the presence of reactive oxygen species and O₂. This peroxy radical reacts with β-Carotene to form a stable β-Carotene

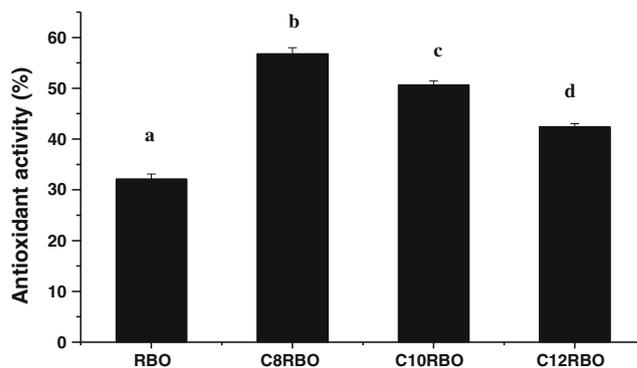


Fig. 10 β-carotene bleaching activity of oil samples (Values are Mean±S.D)

radical and subsequently, the amount of β -Carotene reduces in testing solution. If an antioxidant is present in the testing solution, it reacts competitively with the peroxy radical (Takada et al. 2006). Therefore antioxidant effects are easily monitored by bleaching the colour of a test solution with a spectrophotometer at 470 nm, which is the typical absorbance of β -Carotene. Antioxidant activities of the oil samples were determined by this method. In this study, linoleic acid was selectively oxidized with lipoxygenase. Figure 10 depicts that among all the oils caprylic acid rich RBO showed the highest antioxidative activity and RBO the lowest.

Conclusion

The focus of the present study was to establish the *in vitro* antioxidant property of medium chain fatty acids-rich rice bran oil in comparison to rice bran oil. Three types of MCFA rich rice bran oil were used in this study, namely caprylic acid rich rice bran oil, capric acid rich rice bran oil and lauric acid rich rice bran oil. The antioxidative activity of the oils followed the order Caprylic acid rich rice bran oil > Capric acid rich rice bran oil > Lauric acid rich rice bran oil > RBO. This pattern was consistent with all the antioxidative tests performed in this study. Since the health benefits of dietary antioxidants are well established, the consumption of oils rich in MCFAs can provide health benefits as assessed using various radical scavenging capacities. The increased antioxidative capacity of MCFA rich RBO in comparison to the rice bran oil may be attributed to the decrease in unsaturated fatty acids by inter-esterification with MCFA.

Acknowledgments Financial support obtained from University Grants Commission Dr. D.S. Kothari Fellowship is gratefully acknowledged by the authors.

References

Abd El-Baky HH, El-Baroty GS (2008) Chemical and biological evaluation of the essential oil of Egyptian Moldavian balm. *Int J Essent Oil Ther* 2:76–81

- Alderton WK, Cooper CE, Knochel RG (2001) Nitric oxide synthases: structure, function and inhibition. *Biochem J* 357:593–615
- Arnao MB, Cano A, Acosta M (2001) The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem* 73:239–244
- Cheruvanky R (2000) Bioactives in rice bran and rice bran oil. In *Phytochemicals as Bioactive Agents* CRC Press, Boca Raton, Florida pp 213–240.
- Kleinvelde HA, Hak Lemmers HL, Stalenhoef AF, Demacker PN (1992) Improved measurement of low-density-lipoprotein susceptibility to copper-induced oxidation: application of a short procedure for isolating low-density lipoprotein susceptibility to copper-induced oxidation: application of a short procedure for isolating low density lipoprotein. *Clin Chem* 38:2066–2072
- Lichtenstein AH, Ausman LM, Carrasco W, Gualtieri LJ, Jenner JL, Ordovas JM, Nicolosi RJ, Goldin BR, Schaefer EJ (1994) Rice bran oil consumption and plasma lipid levels in moderately hypercholesterolemic humans. *Atheroscler Thromb* 14:549–556
- MacDonald-Wicks LK, Wood LG, Garg ML (2006) Methodology for the determination of biological antioxidant capacity *in vitro*: a review. *J Sci Food Agric* 86:2046–2056
- Marcocci L, Maguire JJ, Droylefaix MT, Packer L (1994) The nitric oxide-scavenging properties of ginkgo biloba extract EGB 761. *Biochem Biophys Res Comm* 201:748–755
- Marcuse R, Johansson L (1973) Studies on the TBA test for rancidity grading: II. TBA reactivity of different aldehyde classes. *J Am Oil Chem Soc* 50:387–391
- Moore K, Roberts LJ (1998) Measurement of lipid peroxidation. *Free Rad Res* 28:659–671
- Oyaizu M (1986) Studies on product of browning reaction prepared from glucose amine. *Jpn J Nutr* 44:307–315
- Ozsoy N, Can A, Yanardag R, Akev N (2008) Antioxidant activity of Smilax excels L leaf extracts. *Food Chem* 110(3):571–583
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans CA (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Rad Biol Med* 26:1231–1237
- Shyamala BN, Gupta S, Lakshmi AJ, Prakash J (2005) Leafy vegetable extracts- antioxidant activity and effect on storage stability of heated oils. *Innov Food Sci Emer Technol* 6(2):239–245
- Sierra S, Lara-Villoslada F, Olivares M, Jimenez J, Boza J, Xaus J (2005) Increased immune response in mice consuming rice bran oil. *Eur J Nutr* 44:509–516
- Singh RP, Chidambara Murthy KN, Jayaprakash GK (2002) Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. *J Agric Food Chem* 50(1):81–86
- Takada H, Kokubo K, Matsubayashi K, Oshima T (2006) Antioxidant activity of supramolecular water-soluble fullerenes evaluated by β -Carotene bleaching assay. *Biosci Biotechnol Biochem* 70:3088–3093
- Tsuchihashi H, Kigoshi M, Iwatsuki M, Niki E (1995) Action of β -carotene as an antioxidant against lipid peroxidation. *Arc Biochem Biophys* 323:137–147
- Wells JH (1993) Utilization of rice bran and oil in human diets. *Louisiana Agri* 36:4–8