Comparison antioxidant activity of *Tarom Mahali* rice bran extracted from different extraction methods and its effect on canola oil stabilization

Reza Farahmandfar · Maryam Asnaashari ·
Ruhollah Sayyad

Revised: 21 December 2014 / Accepted: 23 December 2014 / Published online: 29 January 2015

© Association of Food Scientists & Technologists (India) 2015

Abstract In this study, *Tarom Mahali* rice bran extracts by ultrasound assisted and traditional solvent (ethanol and ethanol: water (50:50)) extraction method were compared. The total phenolic and tocopherol content and antioxidant activity of the extracts was determined and compared with TBHQ by DPPH assay and β -carotene bleaching method. The results show that the extract from ethanol: water (50:50) ultrasonic treatment with high amount of phenols (919.66 mg gallic acid/g extract, tocopherols (438.4 μ g α -tocopherol/ mL extract) indicated the highest antioxidant activity (80.36 % radical scavenging and 62.69 % β -carotene-linoleic bleaching) and thermal stability (4.95 h) at 120 °C in canola oil. Being high in antioxidant and antiradical potential and high content of phenolic and tocopherol compounds of ethanol: water (50:50) ultrasonic extract caused to evaluate its thermal stability at 180 °C in canola oil during frying process. So, different concentrations of *Tarom Mahali* rice bran extract (100, 800, and 1200 ppm) were added to canola oil. TBHQ at 100 ppm served as standard besides the control. Free fatty acids (FFAs), Peroxide value (PV), carbonyl value (CV), total polar

compounds (TPC) and oxidative stability index (OSI) were taken as parameters for evaluation of effectiveness of *Tarom Mahali* rice bran extract in stabilization of canola oil. Results from different parameters were in agreement with each other, suggesting that 800 ppm of the extract could act better than 100 ppm TBHQ in inhibition of lipid oxidation in canola oil during frying process and can be used as predominant alternative of synthetic antioxidants.

Keywords Canola oil · Lipid oxidation · Rice bran · Stabilization · *Tarom Mahali*

Introduction

Canola oil, due to its high content of polyunsaturated fatty acids (PUFA), is considered better for health than many other vegetable oils, but it is inferior in thermal stability at high temperatures (Fennema 1996). The addition of an antioxidant is required to inhibit lipid oxidation and preserve the color, flavor and nutritive components of oils during storage and frying. During oil degradation, a number of chemical reactions occur in the oil, including oxidation, hydrolysis, and polymerization of unsaturated fatty acids, which change the fatty acid composition of the edible oil and produce volatile and non-volatile oxidation products, dimeric, polymeric, or cyclic substances (Asnaashari et al. 2014a). Indeed, in this process, not only desired components are formed but also compounds with adverse nutritional effects and potential hazards to human health (Eshghi et al. 2014). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used to retard oil oxidation. However, many researchers have reported adverse effects of

Highlights • Considerable improvement of the oxidative stability of canola oil by *Tarom Mahali* rice bran extract
• Better effect of ultrasonic treatment on phenolic content of the *Tarom Mahali* rice bran extract
• Higher antioxidant and antiradical activity of extract in comparison to TBHQ

R. Farahmandfar · M. Asnaashari (✉)
Department of Food Science and Technology, Sari Agricultural
Sciences & Natural Resources University (SANRU), Sari, Iran
P. O. Box 578
e-mail: m.asnaashari@yahoo.com

R. Sayyad
Department of Food Science and Technology, University of Khazar,
Mahmudabad, Iran

synthetic antioxidants, such as toxicity and carcinogenicity. Safety concerns over synthetic antioxidants have led to an increasing interest in identifying naturally antioxidant sources (Asnaashari et al. 2014b). There is remarkable evidence that increasing natural antioxidant intake such as plant polyphenols, flavonoids and vitamin C, can reduce the risk of chronic and degenerative diseases. So several sources of natural antioxidants have been investigated including cereals, legumes and other plants as well as microorganisms (Wang and Lin 2000).

Rice is the main staple food in Iran which also exports to other countries. During rice milling, rice bran is produced as a by-product. Although, it has been recognized as an excellent source of vitamins and minerals, it has been under-utilized as a human food and has traditionally been used in animal foods (Razavi and Farahmandfar 2008). Recently, researches have shown that rice bran may contain even 100 different antioxidants, as new ones are being discovered (Chotimarkorn et al. 2008). Rice bran has a high nutritive value, health beneficial effects such as blood cholesterol lowering, having laxative effect and reducing the incidence of atherosclerosis disease (Zhang et al. 2010). The beneficial components of rice bran comprise sterols, higher alcohols, phenolic compounds, gamma-oryzanol which is a primary plant cell walls and offers some benefits in the cure of nerve imbalance and disorders of menopause, tocopherols, tocotrienols which have beneficial effects such as antioxidant and antibacterial properties and anticancer activities (Butsat and Siriamornpun 2010).

The objective of our research was to investigate the total phenolic and tocopherol contents and antioxidant activity of two solvent extracts from *Tarom Mahali* rice bran obtained by maceration using ethanol and ethanol: water (50:50) as solvent and compare with the same extracts obtained by ultrasonic extraction. Moreover, oxidative stability of canola oil added different concentrations of the extract using primary and secondary oxidation products were also determined.

Materials and methods

Materials

Tarom Mahali was obtained from Amol city, Mazandaran province, Iran during summer season in 2014. All chemical and solvent were provided from Sigma Aldrich (St. Louis, MO) and Merck (Darmstadt, Germany) companies. Folin reagent from Merck (Darmstadt, Germany) and DPPH (2,2-diphenyl-1-picrylhydrazyl) and β -carotene prepared from Sigma Aldrich (St. Louis, MO) were purchased. TBHQ used as standard antioxidant provided from TITRAN.

Methods

Preparation of Tarom Mahali rice bran extract

Tarom Mahali rice bran powder was obtained by milling rice grain in a local grinding mill, followed by sieving to separate grain from rice bran. Rice bran was ground, then passed through 177–297 μm sieves and heated at 100 °C for 15 min to inactivate endogenous lipase. Rice bran powder (10 g) was extracted with ethanol and ethanol: water (50:50) for 12 h in a shaker (LABTRON Ls-100) in 160 rpm at room temperature. The extracts were filtered through Whatman No.1 filter paper and the filtrate was reserved. The residual rice bran powders were further extracted twice with ethanol and ethanol: water (50:50), separately and the extracts were joined before removing part of the solvent under vacuum oven. The residual crude ethanolic and ethanol: water (50:50) of rice bran extracts were weighted and stored at -18 °C under a nitrogen gas stream (Chotimarkorn et al. 2008).

Ultrasound-assisted extraction

The ultrasound-assisted extraction procedure was used for the extraction of *Tarom Mahali* rice bran powder (10 g) with ethanol and ethanol: water (50:50). The mixture was sonicated for 20 min in an ultrasonic bath (Elma s 30 H model, total Power Consumption: 280 W, Heating Power: 200 W, operating at 20 kHz frequency and internal dimensions: $198 \times 106 \times 50$ cm). The temperature was controlled and maintained at 45 °C by circulating water. The extracts were filtered and the remaining steps were similar to those of the previous method (Goli et al. 2005).

Determination of total phenolic content

The total phenolic content was determined spectrophotometrically using Folin-Ciocalteu's reagent according to the method described by Pourmorad et al. (2006). The 0.5 mL of ethanol and ethanol: water (50:50) extracts of *Tarom Mahali* rice bran were added, separately to 2.5 mL of a 10-fold diluted Folin-Ciocalteu reagent and 2 mL of 7.5 % Na_2CO_3 , in a volumetric flask reaching the final volume (50 mL) with purified water. The samples were stored overnight and the spectrophotometric analysis was performed at 765 nm. Results were expressed in milligram of gallic acid per gram of extract.

Determination of total tocopherol content

The total tocopherol content was measured spectrophotometrically according to method described by Wong and

coworkers (1998). A calibration curve of pure α -tocopherol in toluene was performed in a concentration range of 0–240 $\mu\text{g/mL}$. 0.2 g of the extract was dissolved in toluene (5 mL) and 3.5 mL of 2,2'-bipyridine (0.07 %w/v in 95 % aqueous ethanol) and 0.5 mL of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.2 %w/v in 95 % aqueous ethanol) to make a volume by adding 95 % aqueous ethanol. After 1 min, the absorbance at 520 nm using a spectrophotometer (GBC, Cintra 20) was read. The procedure was performed in subdued light. And the results are expressed as microgram of α -tocopherol equivalents in milliliter extract (μg α -tocopherol/mL extract).

Determination of radical scavenging activity

The ability of *Tarom Mahali* rice bran ethanol and ethanol: water (50:50) extracts to scavenge DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radicals was measured by the method described by Lima and coworkers (2006). The samples were reacted with the stable DPPH radical in a methanol solution. After a 30 min incubation period at room temperature under dark condition, the absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (GBC, Cintra 20). Inhibition of free radical DPPH in percent (I %) was calculated as following equation:

$$I (\%) = (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100.$$

$$\text{Inhibition } (\%) = ((\text{control absorbance} - \text{sample absorbance}) / \text{control absorbance}) \times 100$$

Fatty acid composition of canola oil

Fatty acid composition of canola oil was determined by gas–liquid chromatography and was reported in relative area percentages. Fatty acid composition of the canola oil was transesterified into their corresponding FAME by vigorous shaking of a solution of oil in hexane (0.3 g in 7 mL) with 2 mL 7 N methanolic potassium hydroxide at 50–55 °C for 10 min. The FAME were identified using a HP-5890 chromatograph (Hewlett-Packard, CA, USA) equipped with a CP-SIL 88 (Supelco, Bellefonte, PA, USA) capillary column of fused silica, 60 min length 0.22 mm i.d., 0.2 mm film thickness, and a flame ionization detector (FID). Helium was used as carrier gas with a flow rate of 1 mL/min. The oven temperature was maintained at 195 °C, and that of the injector and the detector at 250 °C (Metcalfe et al. 1996). And calculated oxidizability (Cox) value of canola oil was calculated according to Fatemi and Hammond (1980).

Where A blank is the absorbance of the control reaction (containing all reagent except the test compound), and A sample is the absorbance of the test compound. TBHQ was used as a control antioxidant.

β -Carotene/ linoleic acid bleaching assay

Lipid peroxidation inhibition activity of ethanolic and ethanol: water (50:50) extracts of *Tarom Mahali* rice bran were determined using the β -carotene bleaching method (Zhang and Hamauz 2003). According to this method, 5 mg β -carotene was dissolved in 10 mL chloroform (high-performance liquid chromatography grade), then, 600 μL of the solution were mixed with 40 mg of linoleic acid and 400 mg of Tween 40. Chloroform was completely evaporated using rotary vacuum evaporator. Then, 100 mL of distilled water saturated with oxygen was added and the contents shaken vigorously, 2.5 mL of the above solution was transferred to the test tube and 350 μL of each extract (with a concentration of 2 g/L dissolved in their own solvent) was added. All of the above procedures were done for blanks (β -carotene stock solution in addition to the solvents). All samples were put into a water bath for 120 min at 50 °C. The absorbance values of samples were read spectrophotometrically at 470 nm and were taken immediately at zero time and after 120 min. Antioxidant capacity of the extracts was expressed as percentage inhibition:

Determination of oxidative stability index at 120 °C

Canola oil (3 g), was mixed separately with specific concentration (100 ppm) of the ethanol and ethanol: water (50:50) (maceration and ultrasound treatments) of *Tarom Mahali* rice bran extract and 100 ppm of TBHQ as control antioxidant and then exposed to Rancimat (Metrohm model 734, Herisan Switzerland) at 120 °C at an airflow of 15 L/h. Also, during heating process of canola oil containing different concentrations of rice bran extracts (0, 0.08 % and 0.12 %) and TBHQ at specific time interval (0, 4, 8, 12, 16, 20 and 24 h) also OSI was determined at 120 °C. Measuring vessels, electrodes, connecting tubes and glassware were cleaned several times before the experiments.

Determination of oil samples thermal stability at 180 °C

Refined, bleached and deodorized canola oil (2.5 L) containing different concentrations of ethanol: water (50:50) extract of *Tarom Mahali* rice bran (100, 800 and 1200 ppm) and

TBHQ (100 ppm) were placed in a 2.5-L capacity bench-top deep-fryer (Tefal model 1250, Paris, France) and heated at 180 °C for 24 h. Progress of oxidation was monitored by the determination of PV, FFAs, TPC and CV method.

Peroxide value (PV)

The PV of canola oil samples containing different concentrations of the best *Tarom Mahali* rice bran extract and TBHQ were measured spectrophotometrically at 500 nm by UV–VIS instrument (GBC, Cintra 20). The oil samples were mixed in with 9.8 mL chloroform–methanol (7:3 v/v) on a vortex mixer for 2–4 s. Ammonium thiocyanate solution (50 mL, 30 % w/v) and 50 mL of iron (II) chloride solution ([0.4 g barium chloride dihydrate dissolved in 50 mL H₂O]+[0.5 g FeSO₄·7H₂O dissolved in 50 mL H₂O]+2 mL 10 M HCl, with the precipitate, barium sulphate, filtered off to produce a clear solution]) were added, respectively and after adding each of them, the sample was mixed on a vortex mixer for 2–4 s. Then, the absorbance of the sample was read, after 5 min incubation at room temperature. Results were expressed in milliequivalents of oxygen per kilogram of oil (Shantha and Decker 1994).

Free fatty acids (FFAs) determination

The FFAs was determined according to the AOCS (1993) Official Method Cd 3d-63. In this method, 15±0.01 g of each canola oil samples was placed into a 250 mL Erlenmeyer flask and dissolved in 70 mL reagent grade alcohol containing phenolphthalein indicator and then each oil solution was subsequently titrated with the potassium hydroxide solution.

Total polar compounds (TPC) content

The TPC was determined according to Schulte (2004) method. Briefly, silica gel 60 (63–100 µm), dried for 12 h at 160 °C was added five parts of water to 95 parts of it and was shaken vigorously for about 1 min and stay overnight. Then, one gram of the silica gel 60 was compressed and filled between two cotton wool balls into a 5 mL pipette tip. 500 mg of oil sample was pipette into a 5 mL volumetric flask. It was dissolved in 4 mL toluene and then filled with the toluene. Under a well ventilated fume hood, 1 mL of the solution was pipette on top of the pipette tip. After the solution was soaked in, the pipette tip was washed with 1 mL eluent and after soaking in, were added with 7 mL of eluent. After elution (15 min), the end of the tip was washed with 500 µL of toluene. After the solvent was eliminated, weighing TPC, in percentage (w/w) was calculated by the formula of $100(w-w_1)/w$, in which w and w_1 are the sample weight and the weight of nonpolar compounds in milligrams, respectively.

Carbonyl value (CV)

For CV determination of canola oil samples including different concentrations of rice bran extract and TBHQ, one kilogram of 2-propanol with 0.5 g of sodium borohydride was refluxed for 1 h to remove any carbonyl components of solvent. Then, 2,4-dinitrophenylhydrazine (DNPH) (50 g) were dissolved in 100 ml of the solvent including 3.5 ml of 37 % HCL. 0.04–1 g of the oil sample was made by adding the solvent including triphenylphosphine (0.4 mg/mL) to reach 10 mL. Moreover, the solutions of 50–500 µM of 2,4-decadienal in 2-propanol were prepared. Then, standard carbonyl compound solution or oil solution (1 mL) and DNPH solution (1 mL) were mixed in a tube. After that, the tube was heated (20 min, 40 °C) and cooled in water bath after adding 2 % KOH (8 mL). Finally, the absorbance of upper layer after centrifuging (2000×g for 5 min) was read at 420 nm (Endo et al. 2001).

Statistical analysis

All determinations were carried out in triplicate, and data were subjected to analysis of variance (ANOVA). ANOVA analyses were performed according to SPSS software. Significant differences between means were determined by Duncan's multiple range tests; p values less than 0.05 were considered statistically significant.

Results and discussion

The total phenolic content was determined following Folin-Ciocalteu reagent method and results were expressed as gallic acid equivalents (Table 1). The phenolic compounds of *Tarom Mahali* rice bran extract may contribute directly to antioxidative action. As shown in Table 1, ethanol: water (50:50) ultrasonic rice bran extract had the most content of phenolic compounds (919.66 mg/g), followed by ethanol: water (50:50) maceration (909.34 mg/g), ethanol ultrasonic (886.68 mg/g) and ethanol maceration (862.31 mg/g). Indeed, in ethanol: water (50:50) rice bran (*Tarom Mahali*) extraction, content of phenolic compounds increased due to the higher polarity of mixture in comparison to ethanol extraction. In addition, the presence of water as a solvent leads to a decrease in viscosity of mixture, therefore mass transfer was improved. Ethanol: water (50:50) ultrasonic treatment due to the effect of solvent properties on cavitation bubbles can make a significant difference in terms of phenolic compounds with other extraction method. In mixture of ethanol: water (50:50) extraction method, ethanol (higher vapor pressure) produce more bubbles than water (lower vapor pressure) that requires force to collapse the plant tissue during rice bran

Table 1 Total phenolic, tocopherol and anthocyanin content of *Tarom Mahali* rice bran extracts from different extraction methods

Samples	Total polar content ^a	Total tocopherol content ^b	Inhibition (%) radical scavenging	Inhibition (%) in β -carotene-linoleic bleaching
Ethanol maceration	862.31 $\pm$ 31.4 ^b	455.57 $\pm$ 32.73 ^a	54.77 $\pm$ 3.39 ^d	45.06 $\pm$ 1.96 ^d
Ethanol: water (50:50) maceration	909.34 $\pm$ 19.15 ^{ab}	419.29 $\pm$ 22.2 ^a	62.99 $\pm$ 2.67 ^c	52.02 $\pm$ 1.72 ^c
Ethanol ultrasonic	886.68 $\pm$ 35.64 ^{ab}	441.54 $\pm$ 30.04 ^a	65.74 $\pm$ 3.91 ^c	52.85 $\pm$ 3.49 ^c
Ethanol: water (50:50) ultrasonic	919.66 $\pm$ 17.25 ^a	438.4 $\pm$ 41.42 ^a	80.36 $\pm$ 2.76 ^a	62.69 $\pm$ 2.68 ^b
TBHQ	–	–	74.35 $\pm$ 1.09 ^b	86.23 $\pm$ 3.40 ^a

Different letters in the column indicate significant differences ($p < 0.05$)

^a mg gallic acid/g extract

^b μ g α -tocopherol/ml extract

extraction. Moreover, liquid's surface tension is another feature that contributes to the formation of cavitation bubbles. In the liquids with lower surface tension, cavitation bubbles are created more easily, because ultrasonic intensity applied could more easily exceed the surface tension force. So, ethanol: water (50:50) had better performance in extraction phenolic and can better exertion from plant tissue. However, in the case of viscosity, liquids with low viscosity are more effective, because ultrasonic intensity applied, could more easily overcome molecular force of the liquids with low viscosity and also can easily penetrate into plant texture due to low density and high diffusivity. So mixture of ethanol with water in ultrasonic treatment can help to extract more phenolic content of *Tarom Mahali* rice bran extract.

Tocols (tocopherol and tocotrienol) and oryzanol are the main antioxidants present in rice bran. Tocols constitute a series of related benzopyranols that occur in plant tissues and vegetable oils and are powerful lipid-soluble antioxidants that act as vitamin and antioxidant agents. These compounds are only synthesized by plants and other oxygenic photosynthetic organisms, but they are essential components of the diet of animals (Xu et al. 2001). On the basis of total tocopherol content, ethanol maceration with 455.57 μ g/mL acted better than other extraction methods including ethanol: water (50:50) ultrasonic (438.4 μ g/mL), ethanol ultrasonic (441.54 μ g/mL), ethanol: water (50:50) maceration (419.29 μ g/mL). However, there was not significantly different based on total tocopherol content between various extraction methods of rice bran. In this way rice bran due to a unique complex of oryzanols and tocols, may be a good source of compounds for the inhibition of lipid peroxidation.

DPPH radical-scavenging test is one of the fast methods for determination the hydrogen donating potential of chemical substances results their antioxidant activity. When DPPH, encounters proton radical scavengers its purple color fades rapidly as a measurement of absorption at 517 nm. Antioxidant activity of plant extracts including polyphenolic compounds is contributed to their ability to donate hydrogen atoms or electrons and free electrons. DPPH radical scavenging of *Tarom*

Mahali from different extraction methods are presented in Table 1. The antiradical activity increased in the order water ethanol: water (50:50) ultrasonic (80.36 % of radical scavenging inhibition) > TBHQ (74.35 %) > ethanol ultrasonic (65.74 %) > ethanol: water (50:50) maceration (62.99 %) > ethanol maceration (54.77 %). Moreover, Figure 1 presents the dose–response curve for the free-radical scavenging ability of *Tarom Mahali* rice bran extract by the DPPH coloring method. As shown, the absorbance decreased sharply with the increase in the extract concentration to a certain extent, and then levels off with further decrease of extract concentration. Although, DPPH assay is important way to determine the antioxidant activity of the plants extract, it could not provide enough information about antioxidant activities of phenolic extracts in food systems. So, we measured antioxidant activity of the extracts in the water: oil β -carotene/linoleic acid system. According to this method, peroxy radicals are made from linoleic acid oxidation, oxidized unsaturated β -carotene. So when an antioxidants presents in this system, it can diminish β -carotene deterioration. Therefore, the amount of β -carotene decomposed is related to the antioxidant activity of extract. The effect of *Tarom Mahali* rice bran extracts from different

Fig. 1 Radical scavenging activity of *Tarom Mahali* rice bran extract on DPPH, as measured by changes in absorbance at 517 nm

extraction methods on the β -carotene oxidation has been shown in Table 1. It is obvious that the extracts can be able to scavenge free radicals in the heterogeneous medium. So, these extracts may be used as antioxidant retentive in emulsion systems. As shown in Table 1, the ethanol: water (50:50) ultrasound treatment with 62.69 % was the most effective extraction method in preserve the antioxidant activity of the rice bran extract following by ethanol ultrasonic (52.58 %), ethanol: water (50:50) maceration (52.02 %) and ethanol maceration (45.06 %). And TBHQ (86.23 %) as a synthetic antioxidant shows the highest amount of inhibition. Therefore, we focused on the use of ethanol: water (50:50) ultrasonic treatment of *Tarom Mahali* extract due to high amount of total phenolic and tocopherol contents and remarkably radical scavenging and β -carotene-linoleic bleaching inhibition for the stabilization of canola oil.

Fatty acids composition of canola oil

Chemical characteristics of the canola oil used in this study are shown in Table 2. The canola oil was constituted Oleic (65.39 %), linoleic (16.32 %), α -linolenic (7.54 %) and palmitic acids (4.29 %). Among the fatty acids, the highest percentage of the saturated, monounsaturated and polyunsaturated fatty acids were palmitic acid, oleic acid and linoleic acid, respectively. Moreover, the MUFA/PUFA ratio of canola oil was 2.81. This factor was a measure of oil tendency to oxidation. The higher amount of this factor shows the more oxidative stability of oil especially in frying process. Canola

oil was perhaps the edible vegetable oil that was nutritionally well-balanced, based on a low content of SFA, a high content of MUFA, and a ratio of W6 and W3 PUFA. Also, Cox value which is taken as a measure of oil tendency to oxidation was 3.96 in the canola oil. So, antioxidant for inhibiting lipid oxidation is necessary to be added.

Oxidative stability index of canola oil samples including *Tarom Mahali* rice bran extract

Oxidation is a reaction that occurs in the presence of oxygen and is one of the main causes of vegetable oil deterioration during storage. As is known, canola oil is commonly consumed in daily diet as a vegetable oil. The main part of the canola oil is sensitive to oxygen because of the high unsaturated fatty acid content and for that reason the preservation of canola oil is rather difficult. The degree of lipid oxidation can be determined by chemical and physical methods as well as stability tests. Rancimat is an automated instrument that measures the conductivity of low molecular weight fatty acids produced during autooxidation of lipids at higher 100 °C. The principle of the conductivity determination in Rancimat is based on measuring the resistance of the recovered volatile acids (Sun et al. 2011). Addition of antioxidant agents is a common technique to minimize rancidity and unpleasant flavor formation, preserve nutritional quality, retard the occurrence of toxic substances and prolong the shelf life of vegetable oils. Oxidative stability of edible oils as affected by the antioxidative additives has widely been measured by this method. Figure 2. shows the OSI of the canola oil samples as affected by *Tarom Mahali* rice bran extracted by different extraction methods at 120 °C. All samples of *Tarom Mahali* rice bran extracts from different extraction methods significantly improved the OSI of the canola oil compared to control (3.02 h). The highest stabilizing effect among different extraction methods belonged to ethanol ultrasonic extraction with 5.02 h, followed by ethanol: water (50:50) ultrasonic treatment (4.95 h), ethanol: water (50:50) maceration (4.69 h), ethanol maceration extraction method (4.43 h). However, TBHQ (4.84 h) as a control antioxidant shows the highest stabilizing effect in comparison the *Tarom Mahali* rice bran extracts. Indeed, the induction period, IP, is defined as the time before rapid acceleration of lipid oxidation. Antioxidant activity index (AAI) is parameter to evaluate the effectiveness of antioxidant and is determined as the ratio of IP of vegetable oil samples including different concentrations of antioxidant to that of control. Indeed, AAI indicates the possibility of blocking the chain radical process in interaction with radicals, which is responsible for the duration of induction period. The AAI value of samples were 1.66, 1.63, 1.6, 1.55 and 1.46 for ethanol ultrasonic, ethanol: water (50:50) ultrasonic, TBHQ, ethanol: water (50:50) maceration, ethanol maceration, respectively suggesting an appreciable effectiveness of *Tarom*

Table 2 The fatty acid composition of canola oil

Fatty acids (%)	Canola oil
Myristic acid (C14:0)	0.07±0.02
Palmitic acid (C16:0)	4.29±0.83
Stearic acid (C18:0)	2.59±0.04
Palmitoleic acid (C16:1)	0.29±0.03
Oleic acid (C18:1)	65.39±0.62
Linoleic acid (C18:2)	16.32±0.01
α -Linolenic acid (C18:3)	7.54±0.01
Arachidic acid (C20:0)	0.99±0.02
Erucic acid (C22:1)	1.49±0.48
Others	1.03±0.52
Saturated fatty acids (SFA)	7.94±1.03
Monounsaturated fatty acids (MUFA)	67.17±0.53
Polyunsaturated fatty acids (PUFA)	23.86±0.01
PUFA / SFA	3.01±0.07
MUFA / PUFA	2.81±0.05
USFA / SFA	11.46±0.01
C18:2 / C18:3	2.16±0.1
Calculated oxidizability value (Cox value)	3.96±0.01

Fig. 2 oxidative stability index (OSI) of canola oil as affected by different extraction methods of *Tarom Mahali* rice bran extracts at 120 °C and airflow rate of 15 l/h. Means $\pm$ SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$

Mahali rice bran extract on oxidative stability of canola oil. It is obvious that the antioxidative compounds of *Tarom Mahali* rice bran extract will remarkably be able to prevent oxidative reactions under harsh conditions of Rancimat due to high carry-through property (resistance to be destroyed by heat and/or lost through volatilization).

Oxidative stability of canola oil samples including ethanol: water (50:50) ultrasonic extract of *Tarom Mahali* rice bran

To follow the canola oil oxidation rate, the samples were analyzed periodically for PV, FFAs, CV, OSI and TPC, since a single reaction criterion is not enough to account for the oxidative changes of canola oil in presence of different concentrations of *Tarom Mahali* rice bran extract under different conditions.

Hydroperoxides are the primary products of lipid oxidation. It is well known that hydroperoxide have no undesirable flavour, whereas their decomposed products are mostly responsible for rancid off-flavour. In the all canola oil samples, PVs increased from the beginning of the frying progress to the end, showing the progression of oxidation. For the control canola oil samples, the progressive increase in PVs throughout the storage period was significantly ($p < 0.05$) higher than the samples containing different concentrations of *Tarom Mahali* rice bran extract. So that the PVs of control samples increased 455.5 %, whereas 100 ppm of *Tarom Mahali* rice bran extract showed 316.67 % followed by 800 and 1200 ppm of rice bran

extract with 194.4 % and 175 %, respectively. Moreover, TBHQ as a control antioxidant showed 304.34 % increased in PVs during frying process at 180 °C. So, as the concentration of *Tarom Mahali* rice bran extract increased, its antioxidant effect and inhibition of oil oxidation enhanced.

The measurement of FFAs is one of the most important factors to determine the oil quality and economic value. Since fatty acids are more unstable in the free state than when they have been esterified to glycerol, their presence in oils increases the possibility of rancidity. The large amount of FFAs, especially low molecular weight ones which have been released by hydrolysis from the glycerides due to moisture, temperature and lypolytic enzyme reactions, are responsible for undesirable flavours and aromas in oils (Fennema 1996). The changes of FFAs are shown in Table 3. At the three concentrations of *Tarom Mahali* rice bran extract (100, 800 and 1200 ppm) and 100 ppm of TBHQ, the formation of free fatty acids reduced compared to the control canola oil without any additive. So that, the FFAs of control sample increased from 0.5 mg KOH/g oil to 2.8 mg KOH/g oil. whereas the 100 ppm of *Tarom Mahali* rice bran extract enhanced from 0.49 mg KOH/g oil to 1.53 mg KOH/g oil and 800 ppm and 1200 ppm of the extract reached 1.3 mg KOH/g oil and 1.48 mg KOH/g oil, respectively. TBHQ as a control antioxidant also enhanced to 1.67 mg KOH/g oil during 24 h of frying process at 180 °C. Although the amount of FFAs in 100 ppm of *Tarom Mahali* rice bran is slightly lower than two other concentrations. As we expected, the formation of

Table 3 Free fatty acids (FFAs) of the canola oil as affected by the different concentrations of *Tarom Mahali* rice bran extract (0, 100, 800 and 1200 ppm) during the frying process at 180 °C

Time (hour)	Control	Rice bran extract			TBHQ
		100 ppm	800 ppm	1200 ppm	
0	0.5±0.001 ^{gC}	0.49±0.002 ^{gD}	0.46±0.005 ^{gE}	0.52±0.001 ^{gB}	0.59±0.006 ^{gA}
4	0.82±0.02 ^{fA}	0.76±0.001 ^{fC}	0.62±0.008 ^{fE}	0.7±0.009 ^{fD}	0.79±0.001 ^{fB}
8	1.2±0.03 ^{eA}	0.95±0.002 ^{eC}	0.74±0.005 ^{eE}	0.85±0.005 ^{eD}	0.96±0.006 ^{eB}
12	1.5±0.05 ^{dA}	1.19±0.01 ^{dC}	0.94±0.005 ^{dE}	1.07±0.005 ^{dD}	1.21±0.006 ^{dB}
16	1.9±0.01 ^{cA}	1.4±0.02 ^{cB}	1.08±0.009 ^{cD}	1.23±0.01 ^{cC}	1.39±0.01 ^{cB}
20	2.3±0.1 ^{bA}	1.48±0.01 ^{bC}	1.26±0.008 ^{bE}	1.44±0.009 ^{bD}	1.62±0.01 ^{bB}
24	2.8±0.04 ^{aA}	1.53±0.02 ^{aC}	1.3±0.01 ^{aE}	1.48±0.01 ^{aD}	1.67±0.02 ^{aB}

Means ± SD (standard deviation) within a column with the same lowercase letters are not significantly different at $p < 0.05$

Means ± SD within a row with the same uppercase letters are not significantly different at $p < 0.05$

FFAs at higher concentration (800 ppm) reduced in comparison with 100 and 1200 ppm of *Tarom Mahali* rice bran extract. So, 800 ppm of *Tarom Mahali* rice bran extract can remarkably inhibit the canola oil oxidation.

Carbonyl value is a measurement that estimates the content of secondary products of oxidation during frying process. An increase in the CV is consistent with the increase of lipid oxidation. So that, CV of the canola oil increased and reached a maximum value during the frying process and then reduced as a result of further heat treatment. As seen in Table 4, increase of CV in presence of *Tarom Mahali* rice bran extract is slightly lower than canola oil samples without any extract. So that, the CV of the control samples reached 49.2 µmol/g after 24 h of frying. Whereas CV of 100, 800 and 1200 ppm of *Tarom Mahali* rice bran extract after 12 h reached 35.3 µmol/g, 18.01 µmol/g and 28.75 µmol/g, respectively. And after that the content of carbonyl compounds reduced. This was attributed to the decomposition of carbonyl compounds during the prolonged frying period and the formation of new compounds that were not detectable by the CV assay.

100 ppm of TBHQ also can protect canola oil oxidation based on carbonyl value during frying process. So that, the carbonyl content of canola oil samples including TBHQ after 12 h reached 32.3 µmol/g which could significantly prevent canola oil oxidation in comparison with control sample. The results indicated that 100 ppm of *Tarom Mahali* rice bran extract was more efficient to inhibit oil oxidation than 100 ppm of TBHQ.

The results calculated from the linear relationship between the PVs, FFAs, CVs and frying time for canola oil samples including different concentrations of *Tarom Mahali* rice bran extract and 100 ppm of TBHQ are shown in Table 5. The slope of the linear equations (a value), which were considered to be a measure of PV, FFAs and CV decreased as the concentrations of the *Tarom Mahali* rice bran extract enhanced from 100 ppm to 800 ppm and after that the increase of the extract to 1200 ppm lead to increase in linear relationship slope of PVs, FFAs and CVs. So that, the a value of PV changed from 0.059 to 0.044 with increase the extract concentration from 100 ppm to 800 ppm and after that the a value enhanced to 0.049. whereas the a value of the canola oil samples without any additives

Table 4 Carbonyl value (CV) of the canola oil as affected by the different concentrations of *Tarom Mahali* rice bran extract (0, 100, 800 and 1200 ppm) during the frying process at 180 °C

Time (hour)	Control	Rice bran extract			TBHQ
		100 ppm	800 ppm	1200 ppm	
0	9.09±0.1 ^{gBC}	9.1±0.01 ^{fBC}	8.11±0.8 ^{eC}	9.64±0.75 ^{fAB}	10.4±1.01 ^{fA}
4	20.24±0.04 ^{fA}	17.12±0.2 ^{eB}	12.59±0.93 ^{dD}	14.37±1.06 ^{eC}	16.15±1.19 ^{eB}
8	27.03±0.01 ^{eA}	25.69±0.3 ^{eAB}	17.3±1.12 ^{bcD}	21.19±1.65 ^{cC}	23.81±1.86 ^{eB}
12	36.45±1.2 ^{dA}	35.3±0.01 ^{aA}	18.01±3.88 ^{abC}	28.75±1.89 ^{aB}	32.3±1.13 ^{aAB}
16	40.09±2.3 ^{cA}	29.78±1.0 ^{bB}	18.2±0.83 ^{cE}	20.76±0.95 ^{cD}	23.32±1.07 ^{cC}
20	43.03±2.01 ^{bA}	25.03±0.56 ^{bB}	16.24±0.78 ^{cE}	18.53±0.89 ^{dD}	20.83±1.00 ^{dC}
24	49.2±0.62 ^{aA}	23.91±0.43 ^{dC}	21.77±0.97 ^{aE}	24.85±1.1 ^{bC}	27.92±1.25 ^{bB}

Means ± SD (standard deviation) within a column with the same lowercase letters are not significantly different at $p < 0.05$

Means ± SD within a row with the same uppercase letters are not significantly different at $p < 0.05$

Table 5 The results calculated from the linear relationship between the peroxide value (PV), free fatty acids (FFAs), OSI, carbonyl value (CV), polar compounds (PC) and the storage time of canola oil (24 h) at 180 °Cas affected by different concentrations of *Tarom Mahali* rice bran extract and 100 ppm of TBHQ as a control antioxidant

		Control	Rice bran extract			TBHQ
			100 PPM	800 PPM	1200 PPM	
PV = a (Time) + b	a	0.096±0.001 ^a	0.059±0.01 ^b	0.044±0.002 ^c	0.049±0.01 ^d	0.056±0.002 ^c
	b	0.42±0.2 ^a	0.52±0.03 ^a	0.357±0.12 ^a	0.423±0.1 ^a	0.458±0.003 ^a
	R ²	0.98	0.99	0.99	0.99	0.996
FFA = a (Time) + b	a	0.094±0.001 ^a	0.044±0.001 ^c	0.037±0.002 ^c	0.042±0.00 ^d	0.047±0.002 ^b
	b	0.442±0.09 ^c	0.588±0.07 ^a	0.472±0.03 ^{bc}	0.536±0.01 ^{ab}	0.605±0.05 ^a
	R ²	0.994	0.955	0.986	0.986	0.986
OSI = a (Time) + b	a	−0.196±0.001 ^c	−0.125±0.01 ^c	−0.101±0.00 ^a	−0.114±0.02 ^b	−0.13±0.001 ^d
	b	4.98±0.12 ^b	5.94±0.89 ^{ab}	5.618±0.96 ^{ab}	6.387±1.3 ^{ab}	7.205±0.86 ^a
	R ²	0.964	0.98	0.976	0.974	0.976
CV = a (Time) + b	a	1.596±0.01 ^a	0.575±0.00 ^c	0.439±0.003 ^c	0.637±0.03 ^b	0.548±0.03 ^d
	b	12.97±1.36 ^{bc}	16.79±0.36 ^a	10.76±1.08 ^c	13.35±0.84 ^b	15.52±2.001 ^{ab}
	R ²	0.75	0.63	0.733	0.421	0.425
PC = a (Time) + b	a	0.876±0.002 ^a	0.551±0.001 ^d	0.582±0.03 ^b	0.5±0.001 ^c	0.563±0.02 ^c
	b	5.36±0.00 ^d	6.34±0.02 ^c	5.349±0.05 ^c	7.21±0.036 ^b	8.077±0.028 ^a
	R ²	0.97	0.95	0.964	0.926	0.925

Means within a row with the same lowercase letters are not significantly different at $p < 0.05$

was 0.096 and canola oil samples including 100 ppm TBHQ was 0.056. Moreover, the a value calculated from the linear relationship between the FFAs and frying time for canola oil samples decreased in a following order: control ($a=0.094$) > TBHQ ($a=0.047$) > 100 ppm of the extract ($a=0.044$) > 1200 ppm of the extract ($a=0.042$) > 800 ppm of the extract ($a=0.037$). Moreover, a value from linear relationship between the CVs and frying time indicated that as the concentration of *Tarom Mahali* rice bran extract changed increased to 800 ppm, the rate of lipid oxidation slowed down. So that the a value of CVs was 1.596, 0.637, 0.575, 0.548 and 0.539 for control, 1200 ppm of the extract, TBHQ, 200 ppm of the extract and 800 ppm of the extract, respectively. As a result, formation of secondary lipid oxidation products also reduced with added *Tarom Mahali* rice bran extract in canola oil samples. Oxidative stability index in is similar to the active-oxygen method (AOM); however it is faster and more precise. It determined the oxidative stability of canola oil including different concentrations of *Tarom Mahali* rice bran extract during frying process by passing air through a sample under stable temperature. The lipid deterioration lead to volatile organic acids formation which dissolved into dionized water and changing the conductivity of the water. The OSI value is defined as hours needed for the rate of conductivity change to reach a specific value. Slope of linear relationship between the OSIs of canola oil samples including *Tarom Mahali* rice bran extract and frying time indicated that as the concentration of the extract raised to 800 ppm, the rate of oxidative stability decreasing, reduced

from −0.196 for control sample to −0.125 and −0.101 for 100 ppm and 800 ppm of extract in canola oil samples, respectively. However, as the concentration of *Tarom Mahali* rice bran extract increased, the a value enhanced to −0.114. Moreover, the a value of TBHQ as control antioxidant was −0.13. So that, the OSI value of 800 ppm of the extract changed from 5.58 h to 3.03 h after 24 h of frying process, whereas the OSI value of canola oil samples including TBHQ changed from 7.16 h to 3.89 h during 24 h of frying process (data not shown).

The content of total polar compounds is the most predominant indicator for oil quality. Determination of total polar compounds in frying oil provides a reliable measurement on the extent of deterioration in most situations due to its accuracy and reproducibility. Slope of linear relationship between the total polar content of canola oil samples including 100 ppm, 800 ppm and 1200 ppm *Tarom Mahali* rice bran extract and frying time was 0.551, 0.502, 0.5, respectively. Whereas the a value of control canola oil sample was 0.879 and samples added with 100 ppm TBHQ was 0.563. So that, 800 ppm *Tarom Mahali* rice bran extract could remarkably inhibit lipid oxidation.

Conclusion

Increasing the potential hazards of artificial antioxidants for human health and demand for natural ones cause to develop

many researches to substitute natural antioxidant in food stuffs. Our results in this research indicate that *Tarom Mahali* rice bran extract can be used as a source of safe and effective natural antioxidant. In this study we clearly indicate, 100 ppm of this extract in stabilization of canola oil can act as the same effective as synthetic antioxidants. And increasing the extract concentration to 800 ppm enhanced the performance.

References

- American Oil Chemist's Society (1993) Official methods and recommended practices of the American oil chemists society, 4th edn. AOCS Press, Champaign
- Asnaashari M, Farhoosh R, Sharif A (2014a) Antioxidant activity of gallic acid and methyl gallate in triacylglycerols of Kilka fish oil and its oil-in-water emulsion. *Food Chem* 159:439–444
- Asnaashari M, Tajik R, Khodaparast M (2014b) Antioxidant activity of raspberry (*Rubus fruticosus*) leaves extract and its effect on oxidative stability of sunflower oil. *J Food Sci Technol* 1–8
- Butsat S, Siriamornpun S (2010) Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chem* 119:606–613
- Chotimarkorn C, Benjakul S, Silalai N (2008) Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. *Food Chem* 111:636–641
- Endo Y, Li C, Tagiri-Endo M, Fujimoto K (2001) A modified method for the estimation of total carbonyl compounds in heated and frying oils using 2-propanol as a solvent. *J Am Oil Chem Soc* 78:1021–1024
- Eshghi N, Asnaashari M, Haddad Khodaparast MH, Hosseini F (2014) Evaluating the potential of natural curcumin for oxidative stability of soybean oil. *Nat Prod Res* 28:1375–1378
- Fatemi SH, Hammond EG (1980) Analysis of oleate, linoleate and linolenate hydroperoxides in oxidized ester mixtures. *Lipids* 15:379–385
- Fennema OR (1996) Food chemistry. Marcel Dekker Inc, New York
- Goli AH, Barzegar M, Sahari MA (2005) Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. *Food Chem* 92:521–525
- Lima CF, Fernandes-Ferreira M, Pereira-Wilson C (2006) Phenolic compounds protect HepG2 cells from oxidative damage: relevance of glutathione levels. *Life Sci* 79:2056–2068
- Metcalf LC, Schmitz AF, Pellca JR (1996) Rapid preparation of methyl esters from lipid for chromatography analysis. *Anal Chem* 38:514–415
- Pourmorad F, Hosseinimehr SJ, Shahabimajid N (2006) Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr J Biotechnol* 5:1142–1145
- Razavi SMA, Farahmandfar R (2008) Effect of hulling and milling on the physical properties of rice grains. *Int Agrophysics* 22:353–359
- Schulte E (2004) Economical micromethod for determination of polar components in frying fats. *Eur J Lipid Sci Technol* 106:772–776
- Shantha NC, Decker EA (1994) Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J AOAC Int* 77:421–424
- Sun Y, Wang W, Chen H, Li C (2011) Autoxidation of unsaturated lipids in food emulsion. *Food Sci Nut* 51:453–466
- Wang SY, Lin HS (2000) Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J Agric Food Chem* 48:140–146
- Wong ML, Timms RE, Goh EM (1998) Colorimetric determination of total tocopherols in palm oil, olein and stearin. *J Am Oil Chem Soc* 65:258–261
- Xu Z, Hua N, Godber JS (2001) Antioxidant Activity of Tocopherols, Tocotrienols, and γ -Oryzanol Components from Rice Bran against cholesterol oxidation accelerated by 2,2'-Azobis(2-methylpropionamidine) dihydrochloride†. *J Agric Food Chem* 49:2077–2081
- Zhang D, Hamaux Y (2003) Phenolic compounds, ascorbic acid, carotenoids and antioxidant properties of green, red and yellow bell peppers. *J Food Agric Environ* 1:22–27
- Zhang MW, Zhang RF, Zhang FX, Liu RH (2010) Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. *J Agric Food Chem* 58:7580–7587