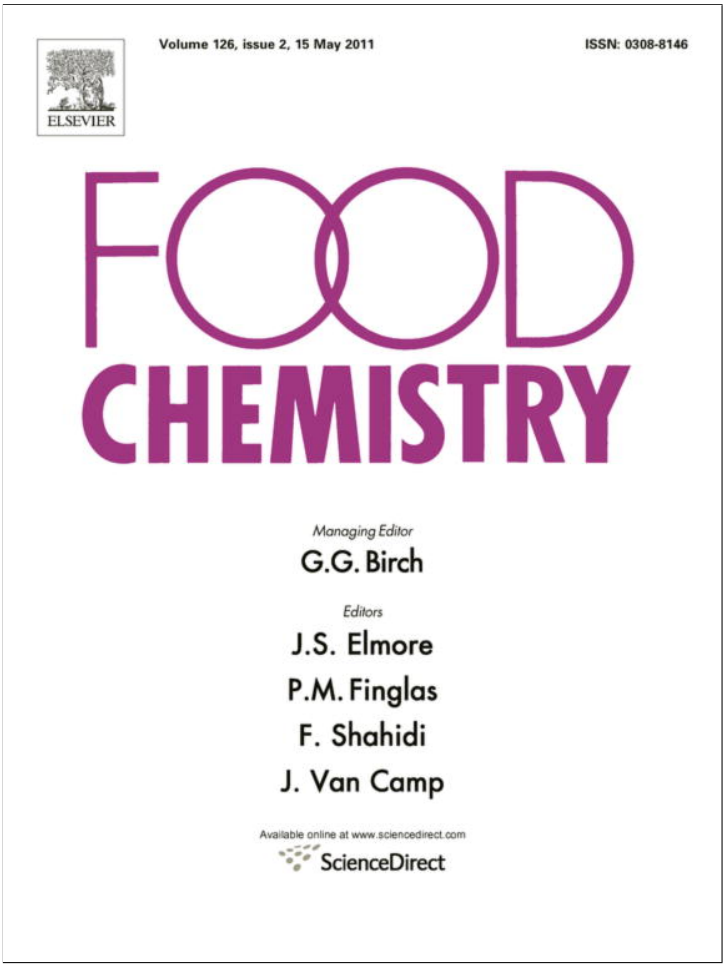

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Antioxidant activity of the fractions separated from the unsaponifiable matter of bene hull oil

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ABSTRACT

The unsaponifiable matter (USM) of bene hull oil were separated into hydrocarbons (7.3%), carotenes (7.1%), tocols (48.4%), linear and triterpenic alcohols (1.7%), methyl sterols (11.2%), sterols (6.2%), and triterpenic dialcohols (18.0%). Regardless hydrocarbons fraction, the whole USM and all fractions had the EC_{50} (mg/ml) value significantly lower than (USM, 0.99; tocols, 1.05; carotenes, 3.93; triterpenoids, 1.74–9.89) that of α-tocopherol (36.7). α-Tocopherol, the whole USM, and all fractions were able to increase significantly the stability of sunflower oil at 50 °C, so that the highest stabilizing effect belonged to the USM fractions with no significant differences amongst them, followed by the whole USM and α-tocopherol. The sunflower oil had an OSI (oil/oxidative stability index) of 3.54 h, which was considerably promoted by some of components (tocols, 4.35; hydrocarbons, 4.43 h; USM, 4.5 h; triterpenic alcohols, 5.1 h; carotenes, 5.49 h). The FRAP and DPPH tests showed nearly similar results.

1. Introduction

Bene (*Pistacia atlantica* subsp. *mutica*) trees grow in large populations and cover an area more than 1,200,000 ha mainly in the western, central and eastern parts of Iran. Its fruits consist of ~24% dark green soft hull, which yield up to ~30% oil (Farhoosh & Tavakoli, 2008). Bene hull oil (BHO) as a new source of highly stable and antioxidative vegetable oils has been recently introduced to the world (Farhoosh, Haddad Khodaparast, & Sharif, 2009). Previous findings showed that the antioxidant activity of BHO was higher than those of the sesame and rice bran oils during frying of sunflower oil (Sharif, Farhoosh, Haddad Khodaparast, & Tavassoli-Kafrani, 2009). The BHO contains about 6.5% USM (Farhoosh et al., 2009), which is considered to be the highest content amongst all common vegetable oils. The results obtained from the different rancidity tests indicated that the frying stability of sunflower oil improved considerably in the presence of the USM extracted from BHO (Farhoosh & Tavassoli-Kafrani, 2010a,2010b). The unsaponifiable constituents of the BHO have been separated into hydrocarbons (HDC), carotenes (CAR), tocopherols and tocotrienols (TAT), linear and triterpenic alcohols (4,4′-dimethyl sterols) (LTA), methyl sterols (4-methyl sterols, MST), sterols (4-desmethyl sterols, STR, no substituent on carbon-4), triterpenic dialcohols (TTD), and triterpenic dialcohols methylesters (TTDM) by means of silica gel thin-layer chromatography (Farhoosh & Tavassoli-Kafrani, 2010a). As can be seen, most of them are particularly important functional compounds which may have the potential to retard the degradation of unsaturated fatty acids in lipid systems. Hence, this study has been undertaken to investigate the antioxidant activity of the fractions separated from BHO compared to that of the whole USM extracted and α-tocopherol as controls.

2. Materials and methods

2.1. Materials

The ripe fruits of bene were collected from the fields of Islamabad in the Ilam province. After drying in the shade at ambient temperature for 48 h, the dark green soft hulls of bene fruits were separated from the dark brown hard shells. The hulls were ground in a grinder. The powders were extracted with *n*-hexane (1:4 w/v) by agitation in a dark place at ambient temperature for 48 h. The solvent was evaporated *in vacuo* at 40 °C to dryness. Refined, bleached, and deodorized sunflower oil (SFO) with no added antioxidant was supplied by Segol (Nishbour, Iran). The bene fruits and oil samples were stored at −18 °C until use. All chemicals and solvents used in this study were of analytical reagent grade and supplied by Merck and Sigma Chemical Companies.

2.2. USM extraction

A mixture of 5 g of the BHO and 50 ml 1 N ethanolic KOH was saponified in a capped flask in an oven for 1 h at 95 °C. After cooling, 100 ml of distilled water was added and mixed. The resulting

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solution was extracted two times with 100 ml diethyl ether. The upper organic layers were combined and washed twice with 75 ml distilled water, once with 100 ml 0.5 N ethanolic KOH, and then 100 ml distilled water until neutrality. The organic layer was then separated and dried over Na_2SO_4. After filtration of this solution, the solvent was evaporated to dryness under vacuum at 45 °C. To purify more effectively, the dry USM was dissolved with chloroform and, after filtration, was evaporated to dryness under vacuum at 45 °C (Lozano, Dhuique Mayer, Bannon, & Gaydou, 1993). The yield of USM extraction was about 5.6 wt.%.

2.3. TLC separation of the USM

A chloroform solution (5%) of the USM (50 mg/plate) was streaked using a thin-layer chromatography (TLC) applicator (CAMAG, Muttenz, Switzerland) along a line at 1 cm from the edges of a 20 cm × 20 cm plate coated with a 0.5-mm layer of silica gel (G), which had been activated for 15 min at 110 °C. The plate was developed in ascending direction for 15 cm with the solvent system *n*-hexane/diethyl ether (7:3 v/v). The developed plate was then dried with a hairdryer, and visualisation of the chromatogram was carried out by spraying a saturated solution of $K_2Cr_2O_7$ in H_2SO_4 (80%) and then carbonating at 130 °C for 25 min. Fractions with the same R_f were carefully scraped from the plate and thoroughly extracted with chloroform; then the extract was filtered through a 0.45-mm membrane filter (Millipore, HVLP) and evaporated to near dryness in vacuo below 40 °C. The residue was weighed to determine the yield of each fraction (Frega & Lercker, 1985).

2.4. HPLC analysis for tocopherols

The content of tocopherols in the oils was determined by using a high-performance liquid chromatograph (WATERS, Allience system, USA) with a Spherisorb column (25 cm × 4 mm i.d., WATERS, USA) packed with silica (5 μm particle size) and a fluorescence detector operating at an excitation wavelength of 290 nm and an emission wavelength of 330 nm (ISO 9936, 1997). The mobile phase used was hexane/isopropanol (98.5:0.5 v/v) at a flow rate of 1 ml/min. Tocopherols in test samples were verified by comparison of retention times with those of reference standards.

2.5. GC analysis for sterols

The composition of the sterol fraction was determined by gas chromatography (GC) using betulin as internal standard (ISO 12228, 1999). The compounds were separated on a SE 54 CB (Macherey–Nagel, Duren, Germany; 50 m long, 0.25 mm ID, 0.25 μm film thickness). Further parameters were as follows: hydrogen as carrier gas, split ratio 1:20, injection and detection temperature adjusted to 320 °C, temperature program, 240–255 °C at 4 °C/min.

2.6. Purification and preparation of the SFO

For the removal of naturally occurring tocopherols from the SFO, 200 g of oil was passed through aluminium oxide 60 (100 g, active, neutral), which had been activated at 200 °C for 3 h immediately before use. The alumina column (25 × 2.5 cm i.d.) and collection vessels were wrapped in aluminium foil, and the oil was drawn through the column by suction without solvent. This procedure was repeated twice to ensure complete elimination of tocopherol homologues (Yoshida, Kondo, & Kajimoto, 1992). The purified SFO (PSFO) was mixed separately with 0.5% (w/w) of the antioxidative oils and 100 ppm of the USMs and α-tocopherol, and then was exposed to the following stability tests.

2.7. DPPH radical-scavenging assay

Various concentrations of toluene sample solutions (1 ml) were mixed with 1 ml of toluenic solution containing DPPH radicals (0.006% w/w). The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm (Ramadan, Kroh, & Morsel, 2003). The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: $\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. The sample concentration providing 50% of radical-scavenging activity (EC_{50}) was calculated by interpolation from the graph of RSA percentage against sample concentration.

2.8. Oven test

The oil samples (10 g) were poured into separate beakers (φ = 38 mm, h = 75 mm) and then placed in an oven at 50 °C. Samples (0.5 ml) were withdrawn at 48-h intervals and subjected to the determination of peroxide value (PV). The spectrophotometric method of the International Dairy Federation as described by Shantha and Decker (1994) was used to determine the PV (thiocyanate method).

2.9. Rancimat test

A Metrohm Rancimat model 743 (Herisau, Switzerland) was used for the oil/oxidative stability index (OSI) measurement. The tests were done with 3-g oil samples at 80 °C and an airflow rate of 15 l/h (Farhoosh, 2007).

2.10. FRAP test

The ferric reducing-antioxidant power (FRAP) test was conducted according to Benzie and Strain (1996). Acetate buffer (0.3 M, pH 3.6) was prepared by dissolving 3.1 g $C_2H_3O_2Na{\cdot}3H_2O$ and 16 ml of acetic acid in 1 l of distilled water. TPTZ (2,4,6-tripyridyl-*S*-triazine) solution was prepared by dissolving 23.4 mg of TPTZ in 7.5 ml of 40 mM HCl solution. Ferric solution (20 mM) was prepared using $FeCl_3{\cdot}6H_2O$. The final working FRAP reagent was prepared freshly by mixing acetate buffer, TPTZ and ferric solutions at a ratio of 10:1:1.

In brief, 900 μl FRAP working reagent was mixed with 90 μl distilled water and was warmed to 37 °C in a water bath. The reagent blank reading was recorded at 595 nm, followed by adding 30 μl of sample solutions (100 mg in 10 ml of *n*-hexane). The absorbance was taken at 595 nm, against the blank solution. A standard curve was prepared using different concentrations of $FeSO_4{\cdot}7H_2O$ (200–2000 μmol/l). All solutions were freshly prepared. The results were expressed in mmol Fe^{2+}/l.

2.11. Statistical analysis

All experiments and measurements were carried out in triplicate, and data were subjected to analysis of variance (ANOVA). ANOVA and regression analyses were performed according to the MStatC and Excel software. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

3. Results and discussion

3.1. Fractionation of the USM

The unsaponifiable constituents of the BHO were separated into the HDC, CAR, TAT, LTA, MST, STR, TTD, and TTDM (Table 1). The TAT was the major constituents (~48%) of the USM. These compounds are particularly important functional constituents of the USM of vegetable oils. They display antioxidant properties and they are active as vitamin E, which makes them particularly important for human health. As can be seen in Fig. 1, β-tocopherol was the predominant tocol isomer (435.8 mg/kg) in the BHO, followed by α-tocopherol (90.3 mg/kg), α-tocotrienol (45.4 mg/kg), δ-tocopherol (6.8 mg/kg), and γ-tocopherol (5.4 mg/kg). The evaluation of antioxidant activity of TAT has always been along with some difficulties, because this activity is frequently influenced by their concentration, the types of oils and fats used as substrates, and the method of evaluation. For example, it has been observed that TAT has substantially the same reactivity with free radicals (Yoshida, Niki, & Noguchi, 2003). Another study indicated no significant differences in the antioxidant activities between TAT in a homogeneous system (Suarna, Hood, Dean, & Stocker, 1993). However, it was reported that α-tocotrienol possessed 40- to 60-fold higher antioxidant activity than α-tocopherol against ferrous iron/ascorbate and ferrous iron/NADPH-induced lipid peroxidation in rat liver microsomes (Serbinova, Kagan, Han, & Packer, 1991), and that α-tocotrienol exhibited greater peroxyl radical scavenging potency than α-tocopherol in liposomal membranes (Suzuki et al., 1993).

The second major constituents of the USM with a total yield of about 37% were the triterpenic compounds (sterolic types and dialcoholic ones) (Table 1). These steroidal phytochemicals contained in vegetable oils are hypocholesterolemic and may also be potent antioxidants. Plant sterols, also called phytosterols, have been reported to include over 250 different sterols and related compounds in various plant and marine materials. The most common representatives are 4-desmethyl sterols (STR), including sitosterol, stigmasterol and campesterol. Sitosterol is the principal sterol in plant materials, but in addition to its 22-dehydro analogue stigmasterol and campesterol, brassicasterol and avenasterol occur in many plant materials. The USM of the BHO contained about 6.2% STR. Fig. 1 shows the sterol composition of the BHO consists of seven compounds with β-sitosterol as the major constituent (746.1 mg/kg). Δ5-(164.1 mg/kg) and Δ7-avenasterols (160.1 mg/kg) were the second predominant STR. The BHO contained lower amounts of stigmasterol (29.9 mg/kg), cholesterol (7.6 mg/kg), brassicasterol (4.3 mg/kg), and campesterol (3.5 mg/kg), respectively. Several findings suggest that phytosterols, such as β-sitosterol, are responsible, at least in part, for preventive effects on the development of diseases due to reactive oxygen species (Vivacons & Moreno, 2005). Moreover, Yoshida and Niki (2003) reported the antioxidant effects of the phytosterols β-sitosterol, stigmasterol, and campesterol, against lipid peroxidation.

The 4-methyl and 4,4′-dimethyl sterols are plant sterol precursors and usually only minor components in most plant sources (Akihisa, Kokke, & Tamura, 1991). The 4,4′-dimethyl sterols of the USM of the BHO were found to be less than 1.7% but surprisingly the 4-methyl sterols constituted a considerable amount (about 11.2%) (Table 1). The 4,4-dimethyl sterols of rice bran oil have recently been the focus of research. Ferulic acid esters of cycloartenol, 24-methylene cycloartanol and cyclobranol, together with some desmethyl steryl ferulic acid esters, are components of a commercial product γ-oryzanol which is produced from rice bran. Amongst commonly used oils, olive and linseed oils contain substantial amounts of dimethyl sterols, with cycloartenol and 24-methylene cycloartanol being the main components. The main monomethyl sterols are usually obtusifoliol, gramisterol, cycloeucalenol and citrostadienol (Piironen, Lindsay, Miettinen, Toivo1, & Lampi, 2000). It has been known since the 1960s that the MST

Table 1
The composition of unsaponifiable matter (USM) of the bene hull oil (BHO).

	R_f	USM fractions (%w/w)
Hydrocarbons (HDC)	0.86	7.28 ± 0.81
Carotenes (CAR)	0.80	7.13 ± 0.40
Tocopherols and tocotrienols (TAT)	0.57	48.43 ± 2.97
Linear and triterpenic alcohols (4,4′-dimethyl sterols) (LTA)	0.40	1.68 ± 0.24
Methyl sterols (4-methyl sterols, MST)	0.33	11.24 ± 1.17
Sterols (4-desmethyl sterols, STR)	0.23	6.23 ± 0.17
Triterpenic dialcohols (TTD)	0.13	9.16 ± 0.93
Triterpenic dialcohol methylesters (TTDM)	0.08	8.85 ± 0.76

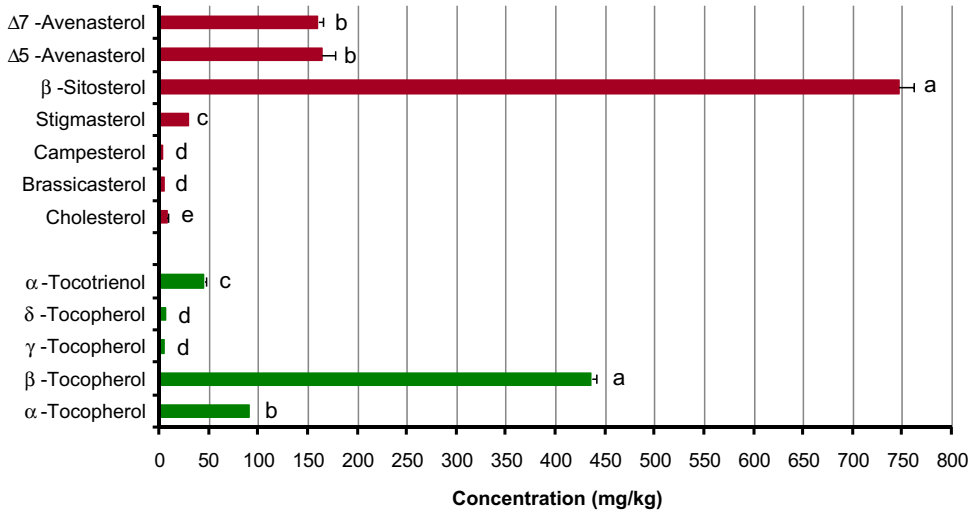

Fig. 1. The contents of tocol compounds and sterols of the bene hull oil (BHO). The columns showing the similar components with the same lowercase letters are not significantly different at $P < 0.05$. Error bars indicate standard deviations.

isolated from the unsaponifiable fraction of wheat germ and corn oils inhibit oxidation of linoleic acid during frying (Maestro Duran & Borja, 1993). The TTD constituted about 18% of the USM of the BHO. Most of studies on this part of USMs have been limited to their presence in olive oils (mainly erythrodiol and uvaol) (Allouche et al., 2009) and our literature review indicated no available data about the antioxidant activity of TTD.

The USM of the BHO contained about 7% HDC. These compounds are mainly linear saturated chains of 15–33 carbon atoms; in food matrices, most of the HDC have an odd number of carbon atoms and are mainly constituted by squalene. It has been indicated that squalene has a potential to retard the degradation of unsaturated fatty acids in lipid systems heated at high temperatures (Malecka, 1991). The CAR constituted about 7% of the USM. These compounds have been shown to protect lipids from free-radical autoxidation by reacting with peroxyl radicals, thereby inhibiting propagation and promoting termination of the oxidation chain reaction (Britton, 1995), and also to be effective quenchers of singlet oxygen during inhibition of photooxidation (Matsushita & Terao, 1980).

3.2. DPPH radical-scavenging assay

Fig. 2 shows the quantities related to the DPPH radical-scavenging activity of the USM of BHO and its fractions. The HDC fraction could not scavenge 50% of the free radicals at the concentrations experimented, and therefore, no EC_{50} value was calculated for it. The USM and all other fractions had the EC_{50} value significantly lower than that of α-tocopherol. The highest DPPH radical-scavenging activity with no statistically difference belonged to the USM (EC_{50} = 0.99 mg/ml) and TAT fractions (EC_{50} = 1.05 mg/ml). The highly DPPH radical-scavenging activity of tocol compounds in non-biological systems has already been demonstrated (Juliano, Cossu, Alamanni, & Piu, 2005); however, considering the high EC_{50} value of the control (α-tocopherol, 36.7 mg/ml), very powerful activity of this fraction can be attributed to the major contribution of β-tocopherol and especially α-tocotrienol (Fig. 1). The EC_{50} value of the LTA fraction was significantly lower (1.74 mg/ml) than those of the other two sterol fractions (6.59 and 9.89 mg/ml for the MST and STR, respectively). It was interesting to find that the DPPH radical-scavenging activity of the sterol fractions increased signifi-

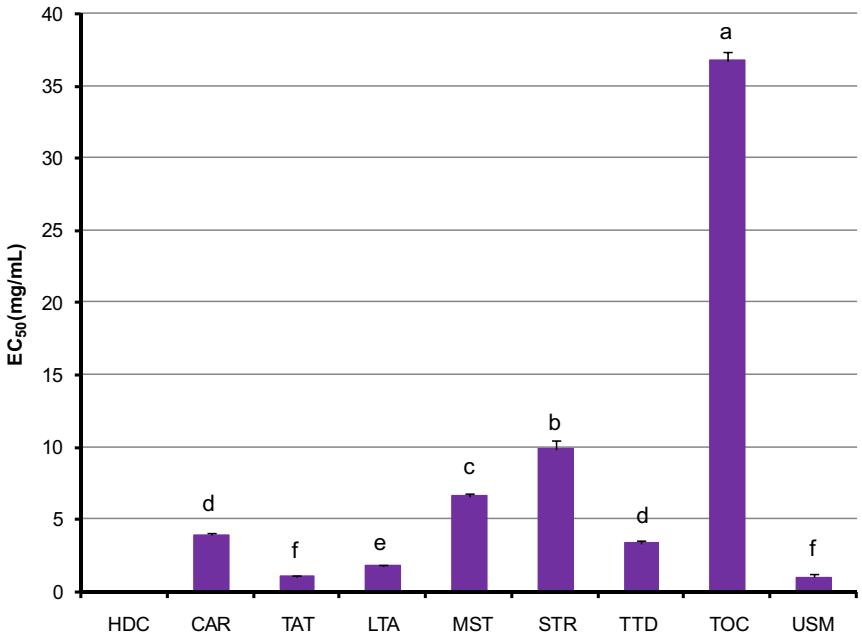

Fig. 2. The sample concentration providing 50% of radical-scavenging activity (EC_{50}) of α-tocopherol (TOC), the unsaponifiable matter (USM) of the bene hull oil (BHO), and the USM fractions of the BHO: HDC, hydrocarbons; CAR, carotenes; TAT, tocopherols and tocotrienols, LTA, linear and triterpenic alcohols (4,4′-dimethy sterols); MST, methyl sterols (4-methyl sterols); STR, sterols (4-desmethyl sterols); TTD, triterpenic dialcohols. Means ± SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$.

Table 2
Changes in the peroxide value (PV, meq O_2/kg oil) of the purified sunflower oil (PSFO) as affected by 100 mg/kg of α-tocopherol (TOC), the unsaponifiable matter (USM) of the bene hull oil (BHO), and the USM fractions of the BHO at 50 °C.

Time (day)	PSFO	TOC	USM	The USM fractions of BHO						
				HDC[a]	CAR[b]	TAT[c]	LTA[d]	MST[e]	STR[f]	TTD[g]
0	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.2	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.0	1.4 ± 0.1
2	70.4 ± 4.4	18.8 ± 0.9	15.2 ± 0.8	36.0 ± 9.6	43.1 ± 0.2	36.7 ± 1.0	37.1 ± 1.1	46.2 ± 0.1	40.2 ± 1.5	42.0 ± 0.3
4	160.8 ± 8.3	86.6 ± 6.2	75.2 ± 2.7	98.4 ± 3.7	107.6 ± 7.5	102.1 ± 4.8	100.0 ± 3.0	113.5 ± 3.6	103.0 ± 1.0	107.7 ± 7.9
6	271.8 ± 15.7	200.8 ± 11.4	138.4 ± 3.8	169.1 ± 1.8	179.8 ± 1.9	184.5 ± 1.5	180.6 ± 1.2	188.0 ± 1.8	180.3 ± 1.2	183.2 ± 1.2
8	609.2 ± 14.8	498.2 ± 16.2	443.7 ± 3.5	284.5 ± 8.6	288.1 ± 2.7	292.7 ± 4.3	285.9 ± 3.3	297.3 ± 4.9	302.1 ± 3.1	290.7 ± 6.4

[a] Hydrocarbons.
[b] Carotenes.
[c] Tocopherols and tocotrienols.
[d] Linear and triterpenic alcohols (4,4′-dimethy sterols).
[e] Methyl sterols (4-methyl sterols).
[f] Sterols (4-desmethyl sterols).
[g] Triterpenic dialcohols.

cantly as the number of methyl groups on carbon-4 increased. Amongst the triterpenic fractions examined, the TTD fraction showed the nearest DPPH radical-scavenging activity to that of LTA (EC_{50} = 3.38 mg/ml). There was observed no statistically significant difference between DPPH radical-scavenging activity of the TTD and CAR fractions (EC_{50} = 3.93 mg/ml). DPPH radical-scavenging activity assay has documented the capacity of carotenoids to quench free radicals by mechanisms that include addition of the radical to the carotenoid, hydrogen abstraction, and/or electron transfer (Moore et al., 2005).

3.3. Oven test

Changes in the PV of the PSFO as affected by the antioxidative compounds added are shown in Table 2. During oxidation, PV of an oil reaches a maximum followed by a decrease at more advanced stages varying according to the oxidative stability of oil. The maximum PV occurs at earlier stages of oxidation in the less stable oils because their hydroperoxides decompose more rapidly. In the present study, the PV of all treatments exponentially increased till the eighth day. Over this time period, an increase of about 435-fold in the PV of the PSFO was observed, which its inverse multiplied by 100 was considered as a stability index (SI_{PV}, 2.30) (Fig. 3). α-Tocopherol, the USM of the BHO, and all fractions separated were able to increase significantly the oxidative stability of the PSFO under the conditions of oven test. The highest stabilizing effect belonged to the USM fractions (SI_{PV} = 4.63–4.92) with no significant differences amongst them, followed by the USM of the BHO (SI_{PV} = 3.16) and α-tocopherol (SI_{PV} = 2.81). As can be seen, the inhibitory effect of the same concentrations of all USM fractions on the formation of primary oxidation products was significantly higher than that of the whole USM containing their different contributions.

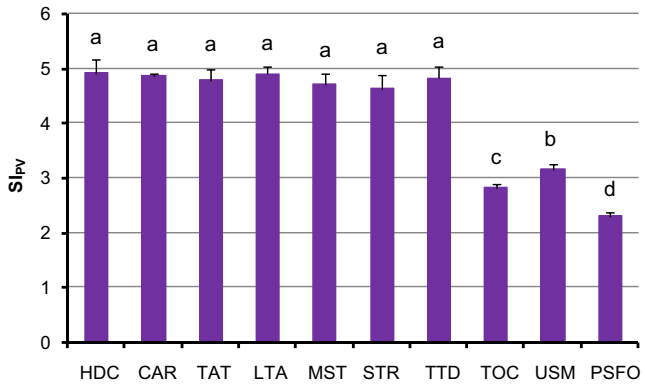

Fig. 3. The inverse of increase amount (folds) in the peroxide value (PV, meq O_2/kg oil) multiplied by 100 (SI_{PV}) for the purified sunflower oil (PSFO) as affected by 100 mg/kg of α-tocopherol (TOC), the unsaponifiable matter (USM) of the bene hull oil (BHO), and the USM fractions of the BHO: HDC, hydrocarbons; CAR, carotenes; TAT, tocopherols and tocotrienols, LTA, linear and triterpenic alcohols (4,4′-dimethy sterols); MST, methyl sterols (4-methyl sterols); STR, sterols (4-desmethyl sterols); TTD, triterpenic dialcohols. Means ± SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$.

3.4. Rancimat test

Fig. 4 shows the OSI of the PSFO as affected by the antioxidative compounds added. Rancimat test has been widely used for the measurement of oxidative stability of edible oils and the capability of different antioxidative compounds. It is obvious that those antioxidative compounds will effectively be able to prevent oxidative reactions under harsh conditions of Rancimat test that have good carry-through properties (resistance to being destroyed by heat and/or lost through volatilisation). The PSFO had an OSI of 3.54 h, which was significantly promoted by some of components experimented. α-Tocopherol could not significantly improve the OSI of the PSFO (3.73 h). The USM of the BHO increased the OSI of the PSFO significantly (4.5 h) but its fractions behaved variously. The highest significant stabilizing effect and also the best carry-through properties amongst the fractions separated belonged to the CAR (5.49 h) and LTA (5.1 h), followed by the HDC (4.43 h) and TAT (4.35 h), the MST (3.93 h), and the TTD (3.68 h) and STR (3.61 h).

3.5. FRAP test

Fig. 5 shows the FRAP quantities of the USM of the BHO and its fractions compared to that of α-tocopherol. The FRAP test is a simple, reproducible, rapid, and inexpensive procedure that measures the ability of antioxidative compounds to reduce the ferric ion Fe^{3+}

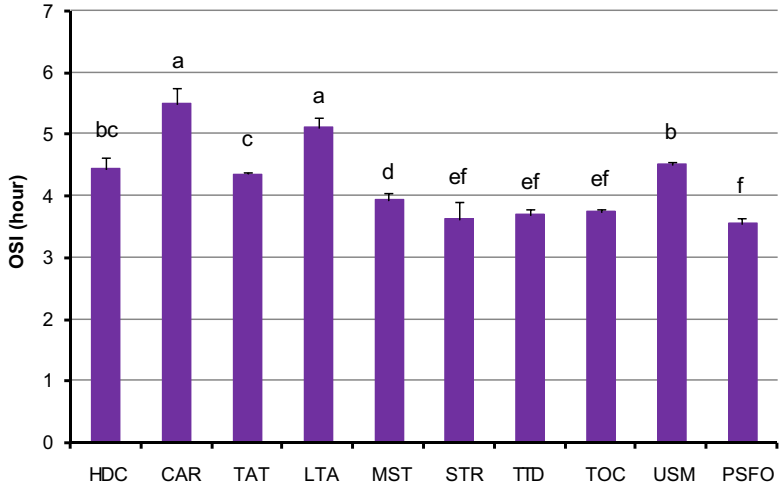

Fig. 4. Oil/oxidative stability index (OSI, Rancimat test) of the purified sunflower oil (PSFO) as affected by 100 mg/kg of α-tocopherol (TOC), the unsaponifiable matter (USM) of the bene hull oil (BHO), and the USM fractions of the BHO at 80 °C and airflow rate of 15 l/h: HDC, hydrocarbons; CAR, carotenes; TAT, tocopherols and tocotrienols, LTA, linear and triterpenic alcohols (4,4′-dimethy sterols); MST, methyl sterols (4-methyl sterols); STR, sterols (4-desmethyl sterols); TTD, triterpenic dialcohols. Means ± SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$.

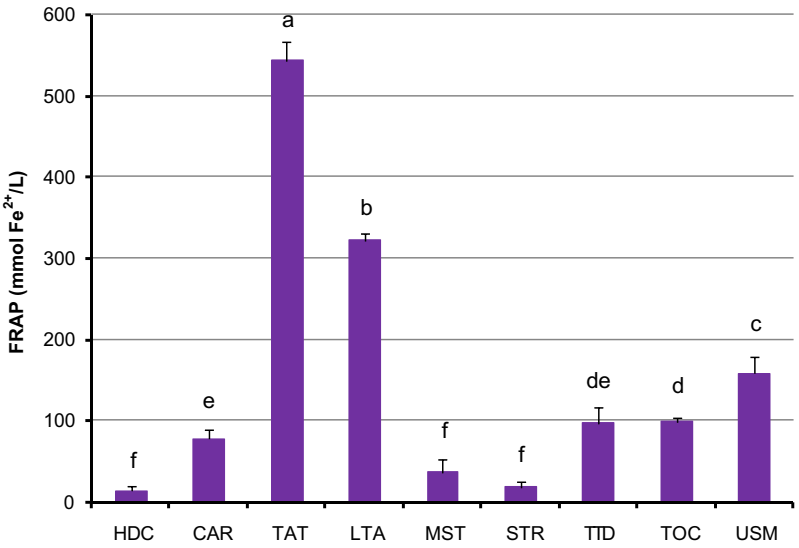

Fig. 5. Ferric reducing-antioxidant power (FRAP) of α-tocopherol (TOC), the unsaponifiable matter (USM) of the bene hull oil (BHO), and the USM fractions of the BHO: HDC, hydrocarbons; CAR, carotenes; TAT, tocopherols and tocotrienols, LTA, linear and triterpenic alcohols (4,4′-dimethy sterols); MST, methyl sterols (4-methyl sterols); STR, sterols (4-desmethyl sterols); TTD, triterpenic dialcohols. Means ± SD (standard deviation) with the same lowercase letters are not significantly different at $P < 0.05$.

to ferrous Fe^{2+}, as a measure of total antioxidant capacity (Prior & Cao, 1999). Many similarities were found to exist between the results of the FRAP test and those of the DPPH radical-scavenging activity assay. The USM of the BHO had a FRAP quantity (158 mmol/l) significantly higher than that of the control (98.2 mmol/l). The highest significant reducing power amongst the USM fractions experimented belonged to the TAT (543.8 mmol/l) and LTA (322.2 mmol/l), respectively. The fractions of TTD (96.5 mmol/l) and CAR (77.5 mmol/l) statistically behaved like α-tocopherol. The fractions of HDC (13.4 mmol/l), STR (17.4 mmol/l), and MST (36.2 mmol/l) indicated the least FRAP quantities amongst the USM fractions.

4. Conclusions

The present study indicated that the USM of the BHO and their fractions can be used as potent natural antioxidants in food systems. The USM contained different contributions of antioxidative fractions which were able to inhibit lipid oxidation effectively by different mechanisms of action. In addition to the fraction of tocopherols and tocotrienols, terpenoid compounds, particularly 4,4′-dimethyl sterols and triterpenic dialcohols, showed antioxidant activities totally better than those of other fractions examined. However, there is no reported information about these fractions in the literature and our research group is attempting to characterise the identity of the most powerful antioxidative constituents amongst them.

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