American Journal of Food Science and Health
Vol. 1, No. 1, 2015, pp. 10-20
http://www.aiscience.org/journal/ajfsh

Bioactives Concentrate from Commercial Indian Niger (*Guizotia Abyssinica* (L.f.) Cass.) Seed and its Antioxidant and Antiradical activity

A. S. Bhatnagar, A. G. Gopalakrishna*

Department of Traditional Foods & Sensory Science, CSIR-Central Food Technological Research Institute, Mysore, India

Abstract

The antioxidant and antiradical activities of bioactives rich niger seed oil (BRNSO) and bioactives concentrate from niger seed oilcake (BCNSOc) were evaluated. BRNSO and BCNSOc contained glycolipids; phospholipids; unsaponifiable matter to the extent of 24.6 and 35.8 g/100g; 9.5 and 23.6 g/100g; 4.3 and 5.5 g/100g, respectively. BRNSO and BCNSOc contained vitamin K_1 (1689.5 and 3225.3 ppm), tocopherols (345.8 and 405.5 ppm), phenolics (2266.2 and 2545.5 ppm), phytosterols (6309.3 and 6834.5 ppm) and carotenoids (181.0 and 301.6 ppm), respectively. BRNSO and BCNSOc also showed strong IC_{50} values of 9.2 and 5.7 mg/mL, respectively. BRNSO (at two different ratios i.e. 1:1 and 1:3) and BCNSOc (at three different levels i.e. 1%, 5% and 10%) were incorporated into commercial refined sunflower oil (SFO) with the aim of improving its oxidative stability (OS) and radical scavenging activity (RSA). Peroxide value (PV) of SFO increased rapidly from 1.2 to 98.2 meq O_2/kg during 45 days of incubation at 37°C while the SFO mixtures with BRNSO and BCNSOc showed better stability as their PV was below 20.0 meq O_2/kg till 30th day of incubation. SFO mixtures with BRNSO and BCNSOc also showed better RSA than SFO. This is probably the first report on bioactives of niger seeds being evaluated for its efficacy in improving the OS and RSA in a bulk oil model system.

Keywords

Indian Niger Seeds (*Guizotia abyssinica* (L.f.) Cass.), Bioactives Rich Niger Seed Oil, Niger Seed Bioactives Concentrate, Oxidative Stability, Radical Scavenging Activity

Received: March 10, 2015 / Accepted: April 1, 2015 / Published online: April 2, 2015

1. Introduction

Niger seed (*Guizotia abyssinica* (L.f.) Cass.) belongs to the compositae family which also include sunflower and safflower seeds. Ethiopia and India are the two major niger seed producing countries in the world (Dutta *et al*., 1994, Dagne and Jonsson, 1997, Ramadan and Moersel, 2002; Marini *et al*., 2003; Geleta *et al*., 2011; Ramadan, 2012). Niger seed is used as a condiment and minor oilseed crop in India amounting to a production of 102,000 tons of seeds and 20,000 tons of oil during 2012-13 (Directorate of Economics and Statistics, 2013). The average oil content of niger seed has been reported to be in the range of 40-44% (Dutta *et al*., 1994, Dagne and Jonsson, 1997, Ramadan and Moersel, 2002; Marini *et al*., 2003; Geleta *et al*., 2011; Ramadan, 2012). Niger seed oil, like sunflower and safflower oils, contains high content of omega-6 PUFA (63-75%) (Dutta *et al*., 1994, Dagne and Jonsson, 1997, Ramadan and Moersel, 2002; Ramadan and Moersel, 2003; Marini *et al*., 2003; Geleta *et al*., 2011; Ramadan, 2012; Bhatnagar and Gopalakrishna, 2013; Bhatnagar and Gopalakrishna, 2014).

Shahidi *et al*., 2003, studied the antioxidant and radical scavenging activities of defatted niger seed and its extracts. They reported that the major active component present was a chlorogenic acid-related compound. They established that

* Corresponding author
E-mail address: aggk_55@yahoo.com (A. G. Gopalakrishna)

chlorogenic acid was dominant in the free phenolic fraction of 2.6 mg/g. The esterified and glycosylated chlorogenic acid on hydrolysis released a substantial amount of caffeic acid (42.8 mg/g). They concluded that defatted niger seed and its extracts can provide a natural source of antioxidants (Shahidi *et al.*, 2003).

Ramadan *et al.*, 2003 reported that the phospholipids of niger seed oil had a strong antioxidant activity because they were involved in synergism with tocopherols, formation of melano-phospholipids, formation of oxygen barrier between oil/air interface and chelation of pro-oxidant metals. They also reported that glycolipids of niger seed oil had a strong antiradical activity due to its reducing sugars [Ramadan, 2012; Ramadan *et al.*, 2003; Ramadan and Moersel, 2004].

Bhatnagar and Gopalakrishna have earlier studied the bioactives composition of niger seed oil (Bhatnagar and Gopalakrishna, 2013; Bhatnagar and Gopalakrishna, 2014). They reported that Indian niger seeds are a rich source of bioactive molecules namely, phytosterols, tocopherols, phenolics, carotenoids, vitamin K_1 (phylloquinone), glycolipids and phospholipids. The inherent bioactive molecules contributed to the oxidative stability and radical quenching ability of niger seed oil. The extractability of these bioactive molecules could be increased by the use of polar solvents. Ethanol proved to be a good solvent for extraction of niger seed oil rich in bioactives (Bhatnagar and Gopalakrishna, 2013; Bhatnagar and Gopalakrishna, 2014).

In the present study, bioactives rich oil and bioactives concentrate from niger seeds is prepared and evaluated for its antioxidant and antiradical activities. The incorporation of niger seed bioactives into commercial refined sunflower oil to enhance its antioxidant and antiradical activity is also attempted. The comparative effect of niger seed bioactives on the antioxidant and antiradical activity of another oil has not been reported so far. The objective of this study was the preparation of bioactives rich oil and bioactives concentrate from niger seeds and their incorporation into commercial refined sunflower oil to improve its antioxidant and antiradical property in comparison to commercial refined sunflower oil added with synthetic antioxidant.

2. Materials and Methods

2.1. Materials

Niger seeds (*Guizotia abyssinica* (L.f.) Cass.) (variety No. 71) commercially traded locally was procured from the local market of Mysore city, Karnataka, India and stored at 4°C until extraction. Commercial refined sunflower oil (without any added synthetic antioxidant) – Sunpure brand, M/s M.K. Agrotech Pvt. Ltd. Mandya, Karnataka, India was procured from the local supermarket of Mysore. Reference standards, α-tocopherol, cholesterol, gallic acid, β-carotene and 2,2-diphenyl-1-picryl hydrazyl free radicals (DPPH) were procured from the Sigma Chemical Co., St. Louis, USA. Reference standard, vitamin K_1 (2-methyl-3-phytyl-1,4-naphthochinon, phylloquinone) was purchased from Merck (Darmstadt, Germany). Reference standard, FAME mix C8-C24 was procured from Supelco, Bellefonte, USA. Tert-Butyl hydro Quinone (TBHQ) was procured from Loba Chemie, Mumbai, India. The extraction solvent, ethanol (77-78°C), used was of commercial grade. The other chemicals and reagents used for analysis were of analytical reagent grade.

2.2. Pilot Scale Preparation of Bioactives Rich Niger Seed Oil (BRNSO)

Niger seeds were cleaned by using a Destoner (Model No. 62, Goldin India Equipment Pvt. Ltd., Vadodara, Gujarat, India). Cleaned niger seeds were flaked to a thickness of 0.5mm by using a flaking machine (Model No. J 6725, Aktiebolaget, Kvarnamaskiner, MALMO, Sweden). Flaked niger seeds (5 Kg x 2) were fed to a batch type Solvent Extraction Unit (ARMFIELD, Model No. FT 29, Armfield Ltd., Ringwood, England) for oil extraction by the standardized procedure. Ethanol (40 L) was used as the extraction solvent. Flaked niger seeds were allowed to soak in ethanol (25 L) for 3h at 35-40°C and miscella (solvent + oil mixture) was collected and transferred into the miscella tank. Later, the soaked niger seeds were continuously purged with fresh ethanol for 5h at a temperature of 45-50°C and the resultant miscella pooled, desolventized and the oil was obtained. The oil extraction of niger seeds by ethanol was done in duplicate. The niger seed oil extracted by using ethanol was labeled as BRNSO.

2.3. Laboratory Scale Preparation of Bioactives Concentrate from Niger Seed Oilcake (BCNSOc)

Niger seed oilcake was obtained by cold pressing (ghani pressing) of fresh, cleaned niger seeds. Briefly, cleaned Niger seeds (12 Kg x 2) were fed to a commercial *Ghani* (traditional Indian oil expeller), Model No. SE PG15, Shyam Engineering Ltd., Rajasthan, India, running at 60 rpm powered by a 3HP motor. Gradual addition of water (500 mL) was done throughout the pressing time to facilitate oil expression. Oil started to ooze out after initial 10 minutes of pressing and was simultaneously filtered and collected in a separate tank at the bottom of Ghani. Gradual formation of hardened cake mass was also observed during pressing. Generation of heat due to friction was observed during pressing, however, the temperature of the seeds and the extracted oil while pressing remained between 50-55°C and 40-45°C, respectively. Pressing was continued for 35-40

minutes till the oil stopped oozing out at the bottom of the ghani which was taken as the end point of the pressing process. Later, the hardened oilcake was removed from the ghani and collected separately. The oilcake obtained from the ghani pressing was ground into a fine powder by using a laboratory grinder. The niger seed oilcake powder (500g x 2) was properly packed into a handmade thimble made out of Whatman No. 1 filter paper. The packed thimble was placed in to the extractor (middle piece) of a customized soxhlet apparatus (1 kg capacity). Hot ethanol (55-60°C) was percolated continuously for 12 h. The condenser and extractor of the soxhlet apparatus was circulated with normal tap water (35-40°C). After 12 h of extraction, the miscella (solvent + extract) was desolventized by flash evaporator (Rotavapor R-215, Buchi Labortechnik AG, Switzerland) and the extract obtained was determined gravimetrically and expressed as g / 100g. The extraction of ground niger seed oilcake powder with ethanol was done in duplicate. The extract obtained from ground niger seed oilcake powder extracted by using ethanol was labeled as BCNSOc.

2.4. Incorporation of BRNSO and BCNSOc into Commercial Refined Sunflower Oil

Commercial refined sunflower oil (SFO) was incorporated with BRNSO and BCNSOc at 100g levels in different proportions. BRNSO being an oil was incorporated to SFO at two different ratios (1:1 and 1:3; wt./wt.). BCNSOc being a semisolid mass was incorporated to SFO at three different proportions (1%, 5% and 10%; wt./wt.). The incorporation of BRNSO and BCNSOc to SFO was done by accurately weighing the constituents in a stoppered flask in the desired ratio and then mixing them well at 100 rpm and 65°C for 30 minutes under vacuum (10 mbar) by using a Buchi Rotavapor (Rotavapor R-215, Buchi Labortechnik AG, Switzerland). The detailed composition of the oil mixtures of SFO with BRNSO and BCNSOc is provided in Table 1.

2.5. Column Chromatography Fractionation of the Lipid Classes

The lipid classes of SFO, BRNSO and BCNSOc were determined by column chromatography as per the procedure by Ramadan and Moersel, 2003. Briefly, a Borosil glass column (30 cm × 20 mm i.d.) was packed with activated silica gel (100–200 mesh; Merck) by applying a slurry of the adsorbent in chloroform (1:5, w/v). Niger seed oils (30 mg/g of adsorbent) were dissolved in 20 mL of chloroform and applied to the column, which was then eluted sequentially with chloroform (for neutral lipids), with acetone (for glycolipids), and with methanol (for phospholipids). The solvent from each fraction was evaporated by rotary evaporation and the percentage of each fraction was determined gravimetrically and expressed as percentage.

2.6. Fatty Acid Composition

SFO, BRNSO and BCNSOc were analyzed for fatty acid composition by using a gas chromatograph. For this purpose, the oil sample was converted into fatty acid methyl esters (FAMEs) by transesterification, using methanolic KOH according to the AOCS Method No: Ce 1-62 (Firestone, 1998). FAMEs were analyzed on a gas chromatograph (GC2010, Shimadzu Co., Kyoto, Japan), equipped with a hydrogen flame ionization detector (FID). A fused silica capillary column (100 m x 0.25 mm i.d.), coated with 0.20 µm SP2560 (Supelco Inc., Bellefonte, PA) was used as the stationary phase. The oven temperature was programmed to initial holding of 5 min at 140 °C and later gradual increase from 140 °C to 240 °C at the rate of 4 °C / min. The injector and FID were kept at 260 °C and the carrier gas, nitrogen was maintained at a flow rate of 40 mL /min. The fatty acids in the samples were identified based on retention time of reference standard FAME mix C8-C24 (Supelco Inc., Bellefonte, USA). The reference standard FAME mix was analyzed under the same operating conditions to determine the peak identity and expressed as relative area percentage.

2.7. Vitamin K_1 (Phylloquinone)

The vitamin K_1 content of SFO, BRNSO and BCNSOc was determined according to Ramadan and Moersel, 2002. Normal-phase liquid chromatography analysis of Vitamin K_1 was performed with a Solvent Delivery Module LC-8A (Shimadzu, Kyoto, Japan). A 250 mm x 4mm column packed with 5 µm diameter LiChrosorb Si 60 (LiChrosorb, Merck Millipore, Merck KGaA, Frankfurter, Darmstadt, Germany) was used for the separation of vitamin K_1. Vitamin K_1 was separated with an isocratic elution by a mixed solvents of Isooctane/ isopropanol (99:1, v/v), and detected at 244 nm with an ultraviolet (UV) detector (Shimadzu, Kyoto, Japan) at room temperature. Oil samples (15-25 mg) dissolved in 10 mL of the mobile phase was injected into the 20 µL sample loop. The solvent flow-rate was maintained at 1 mL / min at a column back-pressure of ca. 85-90 bar. Vitamin K_1 standard was used as external and internal standard for the identification and quantification of Vitamin K_1 in the samples. The vitamin K_1 content of SFO, BRNSO and BCNSOc was expressed in ppm.

2.8. Tocopherols

The tocopherols content of SFO, BRNSO and BCNSOc was determined according to the AOCS Method No: Ce 8-89 (Firestone, 1998) by normal phase HPLC separation on silica column (Lichrosorb Si60 pore size 6nm, pore volume 0.75 mL/g, particle size 7µm, LiChrosorb, Merck Millipore,

Merck KGaA, Frankfurter, Darmstadt, Germany) employing Shimadzu HPLC system consisting of LC-10A pump, injector fitted with 20 µl loop and flourescence detector. The mobile phase was hexane: isopropyl alcohol (99.5:0.5, v/v) at the flow rate of 1mL /min. the excitation wave length of 290 nm and an emission wavelength of 330 nm were kept for the fluorescence detection of all the peaks. Standard α-tocopherol was used as external and internal standards for the identification and quantitation of tocopherols in the samples by comparing the peak retention time and peak area of the standard compound with that of those in the samples. The tocopherols content (as α-tocopherol) in SFO, BRNSO and BCNSOc was expressed in ppm.

2.9. Total Phytosterols by Spectrophotometry

Total phytosterols content of SFO, BRNSO and BCNSOc was determined by the Liberman-Burchard method according to Sabir *et al.*, 2003. Samples (1 g) were weighed and diluted with 10 mL chloroform, and mixed well by vortexing. Aliquots of 3 mL were taken and 2 mL of Liberman-Burchard reagent (0.5 mL of sulphuric acid dissolved in 10 mL of acetic anhydride) was added to them and the final volume was made upto 7 mL by chloroform. Liberman-Burchard reagent reacts with phytosterols to produce a characteristic green color whose absorbance is read at 640 nm. Standard cholesterol solution (1 mg / mL) was prepared, five aliquots of 0.5-2.5 mL were taken and 2 mL of Liberman-Burchard reagent was added and the final volume was made upto 7 mL using chloroform. This mixture was incubated in the dark for 15 minutes and later the absorbance was read at 640 nm. A standard cholesterol curve was generated by plotting absorbance of cholesterol *vs.* amount of cholesterol (µg). The sample absorbance was interpolated on the standard graph and the total phytosterols content (as cholesterol) was calculated and expressed in ppm.

2.10. Total Phenolics by Spectrophotometry

Phenolic compounds were extracted from SFO, BRNSO and BCNSOc using liquid–liquid extraction according to Taga *et al*., 1984, with minor modifications. Twenty grams of sample was dissolved in 50 mL hexane and extracted three times with 30 mL of methanol/water (80:20, v/v). The combined extracts were evaporated to dryness in a rotary evaporator at 40°C. The concentration of total phenolics in the methanolic extract was estimated with Folin-Ciocalteau reagent. A sample aliquot of 100 µL was added to 900 µL of water and 0.5 mL of Folin- Ciocalteau reagent previously diluted with distilled water (1:2 v/v) was added. After 3 min, 1 mL of saturated Na_2CO_3 solution was added; the contents were mixed and the volume was made upto 7 mL with water. The absorbance was measured after 30 min at 750 nm against a reagent blank. Gallic acid served as a standard for preparing the calibration curve (0.05–0.4 mg/mL). The sample absorbance was interpolated on the standard graph and the total phenolics content (as gallic acid) was calculated and expressed in ppm.

2.11. Total Carotenoids by Spectrophotometry

Total carotenoids content of SFO, BRNSO and BCNSOc was determined according to the method of Dauqan *et al.*, 2011, with minor modifications. Samples (100 mg) were weighed and diluted with 10 mL acetone, mixed well by vortexing and their absorbance read at 446 nm (UV-1601, UV visible spectrophotometer, Shimadzu Corporation, Kyoto, Japan) against a blank of pure acetone. Standard β-carotene in acetone (1 mg/mL) solution was prepared and the absorbance of its five aliquots (0.5-2.5 mL) were read at 446 nm. A standard β-carotene curve was generated by plotting absorbance of β-carotene *vs.* amount of β-carotene (µg). The sample absorbance was interpolated on the standard graph and the total carotenoids content (as β-carotene) was calculated and expressed in ppm.

2.12. Radical Scavenging Activity (RSA) and IC_{50} Value

RSA and the presence of hydrogen donors in SFO, BRNSO, BCNSOc and SFO mixtures were examined by reduction of DPPH radicals in toluene. A toluenic solution of DPPH radicals was freshly prepared at a concentration of 10^{-4} M according to Ramadan *et al*., 2003 and Bhatnagar *et al.*, 2009. The samples (50 ± 1 mg) were placed in test tubes and a 4-mL aliquot of DPPH toluenic solution was added and vortexed for 20s at ambient temperature. The decrease in the absorption at 515 nm was measured in a 1-cm quartz cell against a blank of pure toluene without DPPH radicals after 1, 30 and 60 min of mixing using a UV-visible spectrophotometer (model UV-1601, Shimadzu Corporation, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without sample (control). The inhibition percent was calculated using the following equation: Percentage Inhibition = [absorbance of control- absorbance of test sample) /absorbance of control] X 100. IC_{50} value of the oils, expressed as mg / mL, denote the concentration of oil in DPPH toluenic solution required to cause 50% inhibition of DPPH radicals. 5-50 mg of samples were taken and 1-mL aliquot of 10^{-4} M DPPH toluenic solution was added. The reaction mixture was incubated in the dark for 15 minutes and the absorbance was measured at 515 nm in a 1-cm quartz cell. A graph was plotted for percent DPPH remaining against the concentration of oil in DPPH toluenic solution (mg / mL) and IC_{50} value was determined by interpolation of

values on the graph.

2.13. Oxidative Stability

The oxidative stability of SFO + BRNSO/BCNSOc oil mixtures was examined as per the procedure by Bhatnagar *et al.*, 2009, with minor modifications. The oil mixtures (90 g x 2) was placed in screw-capped polyethylene terephthalate (PET) containers (100 mL capacity). The initial headspace of 1.0 cm inside the container was maintained for all the oil samples to be incubated. The oil samples were incubated at 37°C and 55% RH (relative humidity) in a laboratory incubator to observe the oxidative stability over a period of 45 days. In addition, SFO (90 g x 2) and SFO + 200 ppm TBHQ (90 g x 2), as controls, were also incubated simultaneously under similar conditions. Samples (5 g x 2) were withdrawn at every five day interval and analyzed for their peroxide value as per AOCS Method No: Cd 8-53, 1998 (Firestone, 1998).

2.14. Statistical Analysis

The extraction from niger seeds and preparation of SFO mixtures was performed in duplicate, while the estimations were carried out in triplicate making six determinations (n = 6) and the mean ± standard deviation values were reported. Data were analyzed using the statistical program - GraphPad InStat Demo- [DATASET1.ISD], 2014. A two-tailed *p* value was determined to show the significant differences. A significant difference was considered only when the *p* value ≤ 0.05.

3. Results and Discussion

3.1. Oil Yields of Niger Seeds and Niger Seed Oilcake on Extraction with Ethanol

The production yields on extraction of niger seeds and niger seed oilcake by using ethanol as extraction solvent is provided in Table 2. Niger seeds have an oil content of 40.2 g/100g (Bhatnagar and Gopalakrishna, 2013; Bhatnagar and Gopalakrishna, 2014). However, extraction of niger seeds with ethanol does not yield the same oil content. The oil yield of niger seeds when extracted with ethanol came down to 29.7 g/100g (Table 2). Similarly, niger seed oilcake had an oil content of 14.2 g/100g. However, on extraction with ethanol the oil yield of niger seed oilcake was 10.2 g/100g (Table 2). The oil recovery of niger seeds and niger seed oilcake by ethanol was found to be 73.9% and 71.8% respectively (Table 2). The bioactives rich niger seed oil (BRNSO) obtained by extraction of niger seed with ethanol appeared to be a brownish thick liquid, while the bioactives concentrate from niger seed oilcake (BCNSOc) obtained by extraction of niger seed oilcake with ethanol was a dark brown semisolid paste. The unsaponifiable matter of BRNSO and BCNSOc were found to be 4.33% and 5.48%, respectively. The high amount of unsaponifiable matter was probably due to the presence of more amounts of bioactives in BRNSO and BCNSOc which agreed well with our previous reports (Bhatnagar and Gopalakrishna, 2013; Bhatnagar and Gopalakrishna, 2014).

3.2. Lipid Classes of SFO, BRNSO, BCNSOc

The lipid classes of SFO, BRNSO AND BCNSOc is provided in Table 3. SFO, being a commercial refined oil revealed a very high proportion of neutral lipids to the extent of 98.9%. BRNSO and BCNSOc revealed neutral lipid proportions of 65.9% and 40.6%, respectively. The glycolipids and phospholipids present in SFO were 0.9% and 0.2%, respectively. BRNSO and BCNSOc contained glycolipids to the extent of 24.6% and 35.8%, respectively. The phospholipids in BRNSO and BCNSOc were present to the extents of 9.5% and 23.6%, respectively. SFO, being a commercial refined oil had very less glycolipids and phospholipids because refining of oil comprising of dewaxing, degumming, FFA neutralization, water washing, bleaching and deodorization steps strip off its glycolipids and phospholipids and only neutral lipids (especially triacylglycerols) are retained in the oil to give it a crystal clear appearance (Christie, 2012; Leray, 2013). BRNSO and BCNSOc contained more amounts of glyco- and phospho-lipids. The reason for this could be the affinity of ethanol towards carbohydrates (glucose, inositol), amino-alcohols (ethanolamine), polar amino acid (serine), ammonium salt (choline) (Christie, 2012; Leray, 2013; Wan and Wakelyn, 1997). Ethanol, being a polar solvent might have facilitated the extraction of more of polar group (-OH and $-NH_2$) containing glycolipids and phospholipids from the niger seeds and niger seed oilcake. Also, niger seed oilcake, had already got rid of its most of the oil component as being previously cold-pressed for oil. Usually, residual oil present in cold-pressed oilcakes has more amounts of glyco- and phospho-lipids and hence the residual oil subsequently extracted with hexane requires a slightly harsher refining procedure to be converted into a refined oil (Christie, 2012; Leray, 2013; Timothy, 2005).

3.3. Fatty Acid Composition of SFO, BRNSO, BCNSOc

The fatty acid composition of SFO revealed a high content of linoleic acid (C18:2) to the extent of 63.7%. The other fatty acids present in SFO were oleic acid (C18:1), stearic acid (C18:0) and palmitic acid (C16:0) to the extents of 26.4%, 3.9% and 6.0%, respectively (Table 3). The fatty acid

composition of SFO agreed well with the literature (Bhatnagar *et al.*, 2009). The fatty acid composition of BRNSO revealed a high content of linoleic acid (C18:2) to the extent of 69.5%. The other fatty acids present in BRNSO were oleic acid (C18:1), stearic acid (C18:0), palmitic acid (C16:0) and myristic acid (C14:0) to the extents of 15.7%, 6.2%, 8.1% and 0.5%, respectively (Table 3). The fatty acid composition of BCNSOc revealed a high content of linoleic acid (C18:2) to the extent of 70.3%. The other fatty acids present in BCNSOc were oleic acid (C18:1), stearic acid (C18:0), palmitic acid (C16:0) and myristic acid (C14:0) to the extents of 14.4%, 5.4%, 9.4% and 0.5%, respectively (Table 3). The fatty acid composition of BRNSO agreed well with the literature reports (Bhatnagar and Gopalakrishna, 2013; Bhatnagar and Gopalakrishna, 2014), while the fatty acid composition of BCNSOc is probably been reported for the first time. However, the fatty acid composition of BRNSO and BCNSOc were in accordance with the fatty acid composition of niger seed oil being reported in earlier literature reports Dutta *et al*., 1994, Dagne and Jonsson, 1997, Ramadan and Moersel, 2002; Ramadan and Moersel, 2003; Ramadan *et al*., 2003; Marini *et al*., 2003; Ramadan and Moersel, 2004; Geleta *et al*., 2011; Ramadan, 2012). Sunflower and niger seeds belong to same compositae family and hence their fatty acid profile showed similar pattern of fatty acids.

3.4. Bioactives Composition of SFO, BRNSO, BCNSOc

Among bioactive molecules of SFO, BRNSO and BCNSOc, vitamin K_1 (phylloquinone), tocopherols, phenolics, phytosterols and carotenoids were analyzed. The bioactivity of SFO, BRNSO and BCNSOc was assessed by ascertaining their radical scavenging activity towards DPPH radicals and their corresponding IC_{50} values.

3.4.1. Vitamin K_1 (Phylloquinone) Content of SFO, BRNSO, BCNSOc

The vitamin K_1 level is low in most foods i.e. less than 100 ppm. Green and leafy vegetables are the major source of the vitamin K_1. Soybean, cottonseed, and rapeseed oils are important dietary sources of vitamin K_1. Among edible oils, rapeseed oil (ca. 150 ppm) and soybean oil (ca. 130 ppm) are sources of vitamin K_1. Sunflower oil is a very poor source (ca. 10 ppm) of vitamin K_1. The high level of vitamin K_1 is the most unique health promoting characteristic of niger seed oil. Vitamin K_1 is a fat soluble vitamin that functions as a coenzyme and is involved in the synthesis of a number of proteins participating in blood clotting and bone metabolism. Vitamin K_1 reduces the risk of heart disease, kills cancer cells, and enhances skin health and may have antioxidant properties (Ramadan, 2012). The vitamin K_1 content of SFO, BRNSO and BCNSOc is provided in Table 4. Vitamin K_1 was not detected in SFO as SFO is naturally a very poor source of vitamin K_1 and also because of it being commercial refined oil, the refining process might had destroyed whatever little amount of it was present in SFO. BRNSO and BCNSOc contained handsome amounts of vitamin K_1 i.e. 1689.5 and 3225.3 ppm (Table 4). Niger seed is a rich source of vitamin K_1 however, its amount in oil vary depending on the oil extraction mode applied. When we investigated different modes of extraction of niger seed oil we found that cold-pressed niger seed oils (ghani-pressed and hydraulic-pressed) had almost similar amounts of vitamin K_1 i.e. 1344.5 and 1302.4 ppm (data not presented in Table). The hexane-extracted niger seed oil had slightly higher vitamin K_1 level of 1478.6 ppm (data not presented in Table). The ethanol extracted niger seed oil i.e. BRNSO had significantly higher amount of vitamin K_1 i.e. 1689.5 ppm. However the highest level of vitamin K_1 was found in BCNSOc i.e. 3225.3 ppm. This suggests that ethanol is equally efficient in extracting vitamin K_1 as with other bioactives of niger seed. The vitamin K_1 contents reported here agree well with the literature (Ramadan, 2012; Ramadan and Moersel, 2002).

3.4.2. Tocopherols Content of SFO, BRNSO, BCNSOc

Vitamin E or tocopherols which are benzopyranols or methylated tocols are natural antioxidants and integral bioactive molecules of an oil or fat. The primary task of tocopherols is to act as antioxidants to prevent free radical damage to unsaturated lipids or other membrane constituents of the tissues. Tocopherols are powerful antioxidants *in vitro* and *in vivo* (Christie, 2012). The tocopherols content of SFO, BRNSO and BCNSOc is provided in Table 4. The total tocopherols content of SFO was found to be 208.6 ppm with α-tocopherol being the major tocopherol at 166.5 ppm. SFO, being commercial refined oil contained low amount of tocopherols. The chemical nature of tocopherols suggests that they have polar functional group which render them as polar lipids having affinity towards ethanol (Bhatnagar and Gopalakrishna, 2013; Christie, 2012). BRNSO and BCNSOc, being extracted with ethanol contained higher amount of tocopherols. The total tocopherols content of BRNSO was found to be 345.8 ppm with α-tocopherol being the major tocopherol at 276.3 ppm. The total tocopherols content of BCNSOc was found to be 405.5 ppm with α-tocopherol being the major tocopherol at 324.9 ppm (Table 4). The tocopherols contents of SFO and BRNSO agreed well with the literature reports (Bhatnagar *et al*., 2009; Bhatnagar and Gopalakrishna, 2013), while the tocopherols content of BCNSOc is probably been reported for the first time. Alpha-tocopherol comprised roughly about 80% of the total tocopherols present in SFO, BRNSO and BCNSOc.

Sunflower and niger seeds belong to same compositae family and hence their tocopherols profile showed similar pattern of tocopherols.

3.4.3. Phenolics, phytosterols and Carotenoid Contents of SFO, BRNSO, BCNSOc

The total phenolics, phytosterols and carotenoids contents of SFO were found to be 255.5 ppm, 1285.5 ppm and 11.2 ppm, respectively (Table 4). SFO, being commercial refined oil contained low amount of phenolics, phytosterols and carotenoids, as the alkali neutralization followed by water washing, bleaching and deodorization steps involved in refining process removes the phenolics, carotenoids and phytosterols. The chemical nature of phenolic compounds, phytosterols and carotenoids suggests that they have polar functional group which render them as polar lipids having affinity towards ethanol (Bhatnagar and Gopalakrishna, 2013; Christie, 2012). BRNSO and BCNSOc, being extracted with ethanol contained higher amount of phenolics, carotenoids and phytosterols. BRNSO contained high amounts of phenolics, phytosterols and carotenoids at 2266.2 ppm, 6309.3 ppm and 181.0 ppm, respectively (Table 4). BCNSOc also contained high amounts of phenolics, phytosterols and carotenoids at 2545.5 ppm, 6834.5 ppm and 301.6 ppm, respectively (Table 4). The phenolics content of BRNSO and BCNSOc agreed well with earlier literature (Shahidi *et al*., 2003). The phenolics, phytosterols and carotenoids contents of BRNSO agreed well with the previous report (Bhatnagar and Gopalakrishna, 2013) while the phenolics, phytosterols and carotenoids contents of BCNSOc is being reported for the first time.

3.5. Radical Scavenging Activity and IC_{50} Values of SFO, BRNSO and BCNSOc

In our previous study, we have reported that niger seed oil extracted with ethanol had higher amount of glycolipids and phospholipids and also the highest radical scavenging activity was shown by the niger seed oil extracted with ethanol (Bhatnagar and Gopalakrishna, 2014). This also explained and substantiated the role of glycolipids and phospholipids in the antiradical activity of niger seed oil (Bhatnagar and Gopalakrishna, 2013; Bhatnagar and Gopalakrishna, 2014). In the present study, the radical scavenging activity (RSA) of SFO, BRNSO and BCNSOc against DPPH radicals was studied to evaluate their radical quenching ability. BRNSO and BCNSOc had significantly higher RSA ($p \leq 0.05$) compared to SFO (Figure 1). The % Inhibition of SFO, BRNSO, BCNSOc and SFO + TBHQ (200 ppm) at the 1st minute of incubation was 15%, 42%, 55% and 60%, respectively and was found in the order of decreasing prevalence of: SFO + TBHQ (200 ppm) > BCNSOc > BRNSO > SFO. The IC_{50} values (sample concentrations causing 50% reduction in total amount of DPPH radicals) of SFO, BRNSO, BCNSOc was found to be 45.3 mg/mL for SFO, 9.2 mg/mL for BRNSO and 5.7 mg/mL for BCNSOc (Table 4). The RSA of BRNSO and BCNSOc could be attributed to the presence of high amounts of bioactive molecules such as tocopherols, phenolics, vitamin K_1, phytosterols and carotenoids (Bhatnagar and Gopalakrishna, 2013). Apart from these, the presence of glycolipids, phospholipids and maillard reaction products extracted in more amounts by ethanol could also be contributing to the increased RSA of BRNSO and BCNSOc [Ramadan *et al*., 2003; Ramadan and Moersel, 2004; Bhatnagar and Gopalakrishna, 2014]. The RSA of SFO, BRNSO, BCNSOc and their mixtures was found to be in the order of increasing prevalence of SFO, SFO+BCNSOc(1%), SFO+BRNSO(3:1), SFO+BCNSOc(5%), SFO+BRNSO(1:1), SFO+BCNSOc(10%), BRNSO, BCNSOc, SFO+TBHQ(200 ppm).

3.6. Oxidative Stability of SFO Mixtures with BRNSO and BCNSOc

The oxidative stability (OS) of SFO and SFO mixtures with BRNSO, BCNSOc and TBHQ was evaluated by incubating the samples at 37°C and 55% RH. The samples were incubated for a period of 45 days and were analyzed for peroxide value at 5 day interval. The peroxide values of SFO and SFO mixtures with BRNSO, BCNSOc and TBHQ is provided in Figure 2. SFO showed a very rapid formation of hydroperoxides during its incubation period. PV of SFO was highest during the incubation period which increased from 1.2 to 98.2 meq O_2/kg over the period of 45 days of incubation (Figure 2). Addition of TBHQ to SFO resulted in a decrease in initial PV of SFO i.e. from 1.2 to 0.5 meq O_2/kg. SFO + TBHQ (200 ppm) showed very slow formation of hydroperoxides during its incubation period. PV of SFO + TBHQ (200 ppm) was lowest during the incubation period which increased from 0.5 to 6.0 meq O_2/kg over the period of 45 days of incubation (Figure 2). Addition of BRNSO to SFO also resulted in a decrease in initial PV of SFO i.e. from 1.2 to 0.995-0.996 meq O_2/kg. SFO + BRNSO mixtures (1:1 and 3:1) also showed slow formation of hydroperoxides during its incubation period. PV of SFO + BRNSO (1:1) increased from 0.996 to 16.05 meq O_2/kg while the PV of SFO + BRNSO (3:1) increased from 0.995 to 25.08 meq O_2/kg over the period of 45 days of incubation (Figure 2). Addition of BCNSOc to SFO also resulted in a decrease in initial PV of SFO i.e. from 1.2 to 0.996-0.999 meq O_2/kg. SFO + BCNSOc mixtures (1%, 5% and 10%) also showed slow formation of hydroperoxides during its incubation period. PV of SFO + BCNSOc (1%) increased from 0.999 to 28.05 meq O_2/kg while the PV of SFO + BRNSO (5%) increased from 0.996 to 21.07 meq O_2/kg and PV of SFO + BRNSO (10%)

increased from 0.997 to 10.02 meq O_2/kg over the period of 45 days of incubation (Figure 2).

Polyunsaturated fatty acids (PUFA) rich oils like sunflower oil are highly prone to oxidation (Bhatnagar *et al*., 2009) especially when they are refined and stripped off its natural bioactives/antioxidants like tocopherols, phenolics, phytosterols and carotenoids. The incorporation of synthetic antioxidants like TBHQ is a common practise in refined oil industries to provide shelf life to PUFA rich oil. However, prolonged use of refined PUFA rich oils added with synthetic antioxidants may cause serious health issues like cancer (Christie, 2012). Natural bioactives rich oils or natural bioactives concentrates can also provide shelf stability to refined PUFA rich oils. This study made an attempt on improving the shelf life / oxidative stability of PUFA rich refined oil namely, sunflower oil by incorporation of bioactives rich niger seed oil (BRNSO) and bioactives concentrate from niger seed oilcake (BCNSOc). All the SFO mixtures incorporated with BRNSO and BCNSOc showed a much better stability and their PV was below 20.0 meq O_2/kg till the 30th day of incubation (Figure 2). As the rancid flavor and harmful effects of oil becomes apparent only after it crosses the PV of 20.0 meq O_2/kg (Christie, 2012; Gunstone, 2002), it is generally assumed safe to consume oil having a PV of less than 20.0 meq O_2/kg (FSSAI, 2011). Incidently, the PV of SFO shot up to 56.2 meq O_2/kg at the 30th day of incubation which can be very harmful to consume. Among the SFO mixtures with BRNSO and BCNSOc, SFO + BRNSO (1:1) and SFO + BCNSOc (10%) showed the best results as their PV was 16.05 and 10.02 meq O_2/kg till the 45th day of incubation (Figure 2). The OS of SFO and SFO mixtures with BRNSO, BCNSOc and TBHQ was found to be in the order of increasing prevalence of SFO, SFO+BCNSOc(1%), SFO+BRNSO (3:1), SFO+BCNSOc (5%), SFO+BRNSO(1:1), SFO+BCNSOc (10%), SFO+TBHQ (200 ppm).

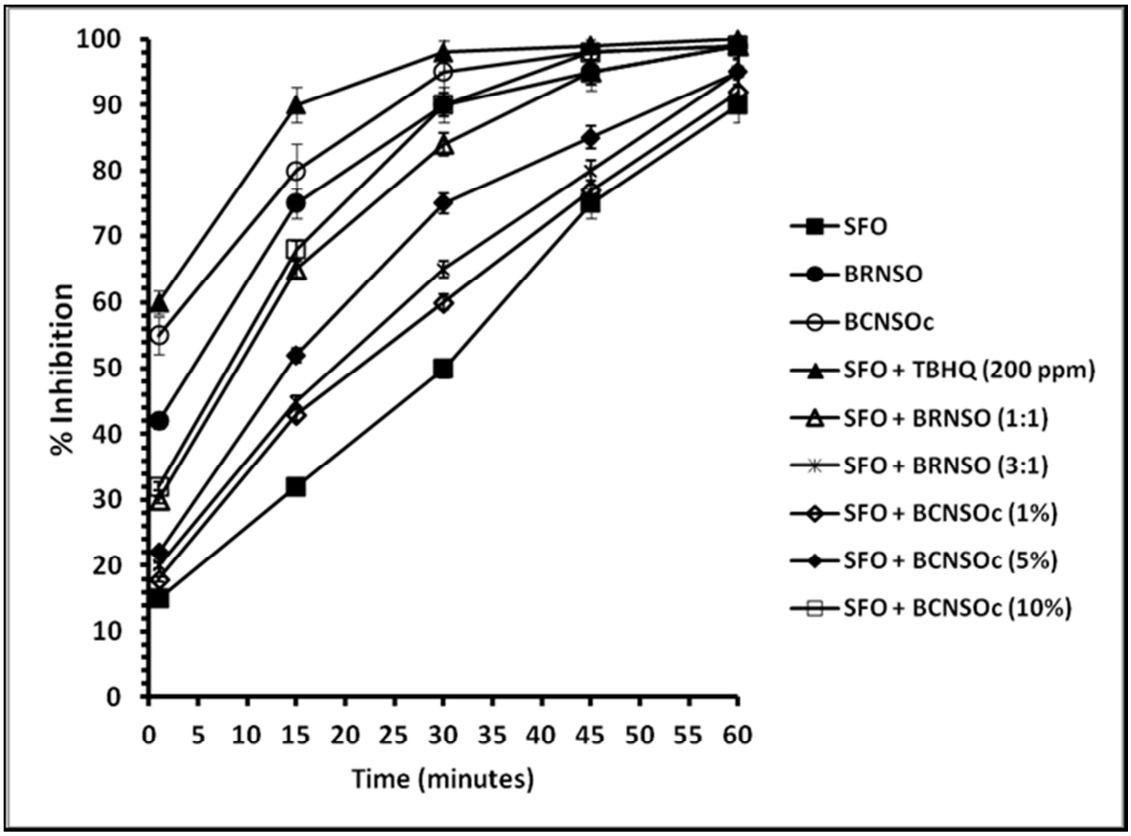

Figure 1. Radical scavenging activity of SFO and SFO mixtures with BRNSO, BCNSOc, TBHQ

SFO, commercial refined sunflower oil; SFO + TBHQ (200 ppm), commercial refined sunflower oil added with TBHQ (200 ppm); SFO + BRNSO (1:1), commercial refined sunflower oil incorporated with bioactives rich niger seed oil in the ratio of 1:1; SFO + BRNSO (3:1), commercial refined sunflower oil incorporated with bioactives rich niger seed oil in the ratio of 3:1; SFO + BCNSOc (1%), commercial refined sunflower oil incorporated with bioactives concentrate from niger seed oilcake at 1% level; SFO + BCNSOc (5%), commercial refined sunflower oil incorporated with bioactives concentrate from niger seed oilcake at 5% level; SFO + BCNSOc (10%), commercial refined sunflower oil incorporated with bioactives concentrate from niger seed oilcake at 10% level

Values are mean ± SD (n = 6)

Table 1. Details of the preparation of SFO mixtures with BRNSO and BCNSOc

Oil mixture	Weight of SFO (g)	Weight of BRNSO (g)	Weight of BCNSOc (g)	Total weight of the oil mixture (g)	Labeled as
1	75.0	25.0	--	100.0	SFO + BRNSO (3:1)
2	50.0	50.0	--	100.0	SFO + BRNSO (1:1)
3	99.0	--	1.0	100.0	SFO + BCNSOc (1%)
4	95.0	--	5.0	100.0	SFO + BCNSOc (5%)
5	90.0	--	10.0	100.0	SFO + BCNSOc (10%)

SFO, commercial refined sunflower oil; BRNSO, bioactives rich niger seed oil; BCNSOc, bioactives concentrate from niger seed oilcake

Table 2. Oil yields of niger seeds and niger seed oilcake on extraction with ethanol

Raw material	Oil content by hexane (g/100g)	Yield by ethanol extraction (g/100g)	Oil recovery by Ethanol extraction (%)	Appearance	Labeling of ethanol extract	Unsaponifiable matter (g/100g) of ethanol extract
Niger seeds	40.2 ± 0.2 [a]	29.7 ± 1.2 [b]	73.9 [1]	Brownish thick liquid	BRNSO	4.33 ± 0.14
Niger seed oilcake	14.2 ± 0.3 [a]	10.2 ± 0.1 [b]	71.8 [1]	Dark brown semisolid paste	BCNSOc	5.48 ± 0.12

[1] With reference to the oil content by soxhlet extraction with hexane i.e. 40.2%
Values are mean ± SD (n = 6)
Values given in rows followed by different alphabetic superscripts are significantly different at $p \leq 0.05$

Table 3. Lipid composition of SFO, BRNSO and BCNSOc

Lipid class (%)	SFO	BRNSO	BCNSOc
Neutral lipids	98.9 ± 0.7 [a]	65.9 ± 1.2 [b]	40.6 ± 1.5 [c]
Glycolipids	0.9 ± 0.1 [a]	24.6 ± 0.8 [b]	35.8 ± 0.9 [c]
Phospholipids	0.2 ± 0.05 [a]	9.5 ± 0.6 [b]	23.6 ± 0.7 [c]
Fatty acid composition (%)			
Myristic acid (C14:0)	--	0.5 ± 0.05 [a]	0.5 ± 0.05 [a]
Palmitic acid (C16:0)	6.0 ± 0.2 [a]	8.1 ± 0.1 [b]	9.4 ± 0.2 [c]
Stearic acid (C18:0)	3.9 ± 0.1 [a]	6.2 ± 0.1 [b]	5.4 ± 0.2 [c]
Oleic acid (C18:1)	26.4 ± 0.1 [a]	15.7 ± 0.2 [b]	14.4 ± 0.2 [c]
Linoleic acid (C18:2)	63.7 ± 0.3 [a]	69.5 ± 0.2 [b]	70.3 ± 0.2 [b]

SFO, commercial refined sunflower oil; BRNSO, bioactives rich niger seed oil; BCNSOc, bioactives concentrate from niger seed oilcake
Values are mean ± SD (n = 6)
Values given in rows followed by different alphabetic superscripts are significantly different at $p \leq 0.05$

Table 4. Bioactive molecules and IC_{50} values of SFO, BRNSO and BCNSOc

Bioactive Molecules	SFO	BRNSO	BCNSOc
Vitamin K_1 (ppm)	Nd	1689.5 ± 5.5 [a]	3225.3 ± 4.8 [b]
α-tocopherol (ppm)	166.5 ± 2.5 [a]	276.3 ± 3.2 [b]	324.9 ± 3.5 [c]
Total tocopherols (as α-tocopherol) (ppm)	208.6 ± 4.0 [a]	345.8 ± 4.6 [b]	405.5 ± 5.5 [c]
Total Phenolics (ppm)	255.5 ± 5.0 [a]	2266.2 ± 17.0 [b]	2545.5 ± 13.0 [c]
Total Phytosterols (ppm)	1285.5 ± 12.0 [a]	6309.3 ± 9.5 [b]	6834.5 ± 8.0 [c]
Total Carotenoids (ppm)	11.2 ± 0.6 [a]	181.01 ± 3.0 [b]	301.6 ± 4.0 [c]
IC_{50} value (mg/mL)	45.3 ± 0.2 [a]	9.2 ± 0.6 [b]	5.7 ± 0.4 [c]

SFO, commercial refined sunflower oil; BRNSO, bioactives rich niger seed oil; BCNSOc, bioactives concentrate from niger seed oilcake
Nd, not detected
Values are mean ± SD (n = 6)
Values given in rows followed by different alphabetic superscripts are significantly different at $p \leq 0.05$

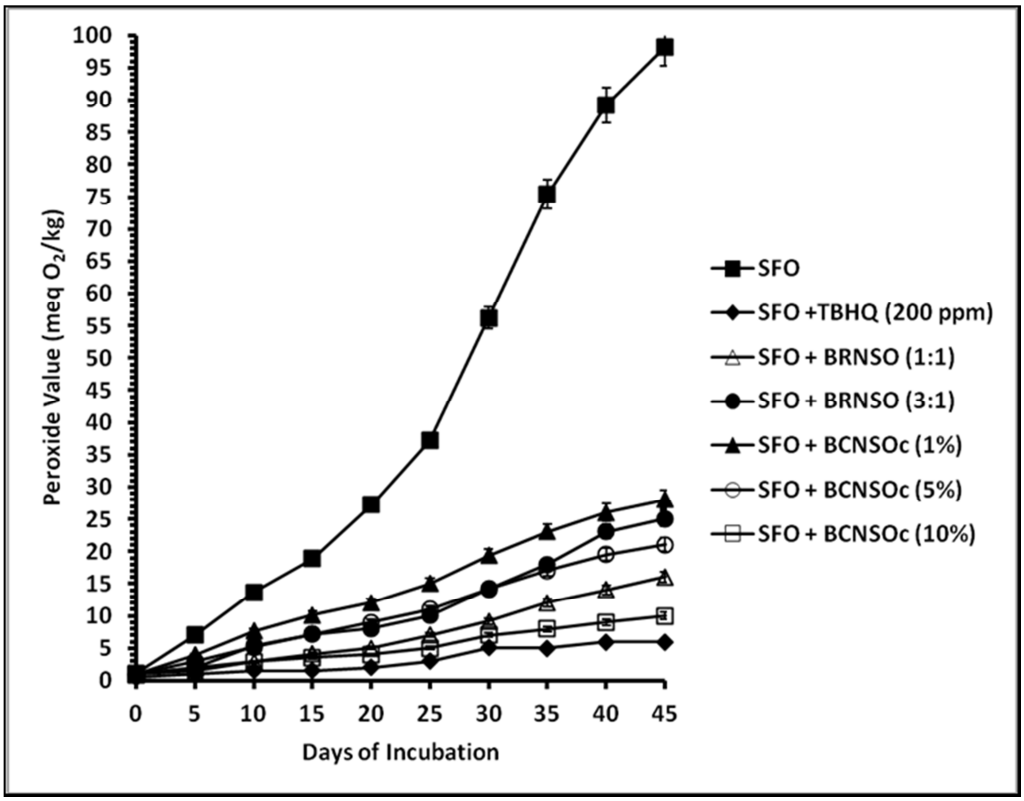

Figure 2. Oxidative stability of SFO and SFO mixtures with BRNSO, BCNSOc, TBHQ

SFO, commercial refined sunflower oil; SFO + TBHQ (200 ppm), commercial refined sunflower oil added with TBHQ (200 ppm); SFO + BRNSO (1:1), commercial refined sunflower oil incorporated with bioactives rich niger seed oil in the ratio of 1:1; SFO + BRNSO (3:1), commercial refined sunflower oil incorporated with bioactives rich niger seed oil in the ratio of 3:1; SFO + BCNSOc (1%), commercial refined sunflower oil incorporated with bioactives concentrate from niger seed oilcake at 1% level; SFO + BCNSOc (5%), commercial refined sunflower oil incorporated with bioactives concentrate from niger seed oilcake at 5% level; SFO + BCNSOc (10%), commercial refined sunflower oil incorporated with bioactives concentrate from niger seed oilcake at 10% level
Values are mean ± SD (n = 6)

4. Conclusions

The present study suggests that incorporation of niger seed bioactives into commercial refined sunflower oil is of great utility in terms of antioxidant and antiradical activity. BRNSO and BCNSOc, despite having higher linoleic acid contents of 69.5 and 70.3% than SFO (63.7%) could enhance the oxidative stability of SFO. This shows that niger seed bioactives namely, phytosterols, tocopherols, phenolics, carotenoids, vitamin K_1 (phylloquinone), glycolipids and phospholipids contributed in enhancing the antioxidant and antiradical activity of commercial refined sunflower oil. Commercial refined sunflower oil enriched by niger seed bioactives, especially, vitamin K_1 (phylloquinone) exhibited enhanced antioxidant and antiradical activity as compared to commercial refined sunflower oil as such. BRNSO and BCNSOc may partially replace synthetic antioxidants for providing stability to PUFA rich refined oils. This is probably the first report on bioactives of niger seeds being proved effective in improving the oxidative stability in a bulk oil model system.

Acknowledgements

The authors are thankful to Prof. Ram Rajasekharan, Director, CSIR-CFTRI, Mysore, for providing infrastructural facilities to carry out this work. One of the authors, Ajit Singh Bhatnagar is thankful to CSIR for the grant of a Senior Research Fellowship for his Ph.D. program.

References

[1] Bhatnagar AS, Gopalakrishna AG. 2013. Effect of extraction solvent on oil and bioactives composition of commercial Indian niger (*Guizotia abyssinica* (L.f.) Cass.) seed. *J. Am. Oil Chem. Soc.* 90, 1203-1212.

[2] Bhatnagar AS, Gopalakrishna AG. 2014. Lipid classes and subclasses of cold-pressed and solvent-extracted oils from commercial Indian niger (Guizotia abyssinica (L.f.) Cass.) seed. *J. Am. Oil Chem. Soc.* 91, 1205-1216.

[3] Bhatnagar AS, Prasanth Kumar PK, Hemavathy J, Gopalakrishna AG. 2009. Fatty acid composition, oxidative stability, and radical scavenging activity of vegetable oil blends with coconut oil. *J. Am. Oil Chem. Soc.* 86(10), 991-999.

[4] Christie WW. 2013. The Lipid Library. http://lipidlibrary.aocs.org, (Accessed on 18th November 2013).

[5] Dagne K, Jonsson A. 1997. Oil content and fatty acid composition of seeds of Guizotia Cass. (Compositae). *J. Sci. Food. Agri.* 73, 274-278.

[6] Dauqan E, Sani HA, Abdullah A, Muhamad H, Top AGM. 2011. Vitamin E and beta carotene composition in four different vegetable oils. *Am. J. App. Sci.* 8, 407- 412.

[7] Directorate of Economics and Statistics, Department of Agriculture and Cooperation. Based on 4th Advance Estimates released by Ministry of Agriculture as on 24.09.2013. http://www.eands.dacnet.nic.in (Accessed on 10.02.2014).

[8] Dutta PC, Helmersson S, Kebedu E, Appelqvist LA. 1994. Variation in lipid composition of niger seed (*Guizotia abyssinica* Cass.) samples collected from different regions in Ethiopia. *J. Am. Oil Chem. Soc.* 71, 839-843.

[9] Firestone D. 1998. Official methods and recommended practices of the American Oil Chemists Society, AOCS method no. Ai 2-75, AOCS method no. Ca 5a-40, AOCS method no. Cd 8-53, AOCS method no. Cd 3c-91, AOCS method no. Cd 1-25, AOCS method no. Ca 6a-40, AOCS method no. Ce 1-62, AOCS method no. Ce 5b-89, AOCS method no. Ce 8-89. 5th Ed. AOCS press, 1608, Broadmoore drive Champaign, Illinois-61826, USA.

[10] FSSAI (Food Safety and Standards Authority of India), Ministry of Health and Family Welfare, Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 1st August, 2011. Chapter 2, Food Product Standards, 2.2.1 (13) Niger seed oil. Part III-Sec.4, The Gazette of India: Extraordinary. pp. 324.

[11] Geleta M, Stymne S, Bryngelsson T. 2011. Variation and inheritance of oil content and fatty acid composition in niger (*Guizotia abyssinica*). *J. Food Comp. Anal.* 24, 995-1003.

[12] Graphpad instat demo-[DATASET1.ISD]. Graphpad Software Inc. 11452, EI Camino Real, #215, San Digeo, 92130 USA. www.graphpad.com (Accessed on Jan. 9, 2012)

[13] Gunstone FD (ed.). 2002. Vegetable oils in food technology: composition, properties and uses. Blackwell publishing, CRC Press, FL, USA. ISBN:1-84127-331-7. pp. 62, 103, 162, 216, 284.

[14] Leray C. 2013. Cyberlipid Center. http://www.cyberlipid.org, (Accessed on 20th November 2013).

[15] Marini F, Magrì AL, Marini D, Balestrieri F. 2003. Characterization of the lipid fraction of Niger seeds (*Guizotia abyssinica* Cass.) from different regions of Ethiopia and India and chemometric authentication of their geographical origin. *Eur. J. Lipid Sci. Technol.* 105, 697–704.

[16] Ramadan MF, Kroh LW, Moersel JT. 2003. Radical scavenging activity of black cumin (Nigella sativa L.), coriander (Coriandrum sativum L.), and niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. *J. Agri. Food Chem.* 51, 6961-6969.

[17] Ramadan MF, Moersel JT. 2002. Direct isocratic normal-phase assay of fat-soluble vitamins and beta-carotene in oilseeds. *Eur. Food Res. Technol.* 214, 521–527.

[18] Ramadan MF, Moersel JT. 2002. Proximate neutral lipid composition of niger (*Guizotia abyssinica* Cass.) seed. *Czech. J. Food Sci.* 20, 98–104.

[19] Ramadan MF, Moersel JT. 2003. Determination of the lipid classes and fatty acid profile of niger (*Guizotia abyssinica* Cass.) seed oil. *Phytochem. Anal.* 14, 366-370.

[20] Ramadan MF, Moersel JT. 2004. Oxidative stability of black cumin (Nigella sativa L.), coriander (Coriandrum sativum L.), and niger (*Guizotia abyssinica* Cass.) crude seed oils upon stripping. *Eur. J. Lipid Sci. Technol.* 106, 35-43.

[21] Ramadan MF. 2012. Functional properties, nutritional value and industrial applications of Niger Oilseeds (*Guizotia abyssinica* Cass.). *Cri. Rev. Food Sci. Nutr.* 52, 1-8.

[22] Sabir SM, Hayat I, Gardezi, SDA. 2003. Estimation of sterols in edible fats and oils. *Pak. J. Nutr.* 2(3), 178-181.

[23] Shahidi F, Desilva C, Amarowicz R. 2003. Antioxidant activity of extracts of defatted seeds of niger (*Guizotia abyssinica*). *J. Am. Oil Chem. Soc.* 80, 443–450.

[24] Taga MS, Miller EE, Pratt DE. 1984. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.* 61, 928–993.

[25] Timothy G. Kemper. 2005. Oil Extraction, Evolution of oil extraction, Bailey's Industrial Oil and Fat Products, Sixth Edition, Six Volume Set. Edited by Fereidoon Shahidi. John Wiley & Sons, Inc pp. 57-60.

[26] Wan PJ, Wakelyn PJ. 1997. Technology and solvents for extracting oileeds and nonpetroleum oils. ISBN: 0935315810, AOCS Press, Champaign, Illinois, USA. pp. 186, 189, 192, 193.