Physico-chemical Characterization, Profiling of Total Lipids and Triacylglycerol Molecular Species of Omega-3 Fatty Acid Rich \textit{B. arvensis} Seed Oil from India

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Abstract: \textit{Buglossoides arvensis} is indigenous to India and its seed oil is rich in unique and nutritionally important omega-3 fatty acid namely, stearidonic acid (SDA). It is a non-conventional oil seed plant and needs to be agronomically adapt for commercial utilization. In the present study, oil extracted from the agronomically adapted high yielding cultivar of \textit{B. arvensis} seeds was analyzed for its oil content, fatty acid (FA) composition, physico-chemical characteristics, total lipids and triacylglycerol molecular species. The oil content, peroxide, acid, iodine, $p$-anisidine values and tocopherol content of the oil were 18.53\% (w/w), 2.06 meq of active oxygen / kg of oil, 2.55 mg KOH/g oil, 217.2 g I$_2$/100g oil, 10.7 and 774.8 mg/kg oil respectively. Oxidative stability as determined by the induction period was found to be 3.1 h.

Polyunsaturated fatty acid (PUFA) content of the oil was 81.3\% (of total FA), comprising of $\alpha$-linolenic acid (ALA; 48.5\%), SDA (18.6\%), linoleic acid (LA; 10.3\%) and $\gamma$-linolenic acid (GLA; 3.9\%). Profiling of lipid classes showed neutral lipids (89.3\%, w/w) as most abundant lipid class followed by glycolipids (7.4\%, w/w) and phospholipids (3.3\%, w/w). High resolution mass spectrometric analysis of triacylglycerol (TAG) molecular species showed TAGs with C54 carbons in the acyl chain as most abundant. Positional distribution analysis showed GLA and SDA predominantly at the $sn$-2 position of triacylglycerol. FTIR analysis revealed common characteristics molecular features similar to PUFA rich oils. Overall, the results suggest that \textit{B. arvensis} seed oil is an excellent $\omega$3-$\omega$6-$\omega$3 or ALA-SDA-GLA source for food and nutraceutical industries.

Key words: \textit{Buglossoides arvensis}, polyunsaturated fatty acids, stearidonic acid, $sn$-2 fatty acids, oxidative stability, tocopherols

1 Introduction

Oils and fats are an essential part of human nutrition. Consumption of foods rich in cholesterol, saturated fat and trans-fat have been related with increased risk of chronic diseases whereas intake of foods rich in omega-3 polyunsaturated fatty acids ($\omega$-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been correlated with their prevention$^{1,2}$. Further, $\omega$-3 PUFA plays a significant role in embryo development and brain function$^3$.

Marine oils (fish and algae) and vegetable oils are the major sources of PUFA. Increased consumption of $\omega$-3 PUFA has placed a significant demand on the marine ecosystem which cannot be met sustainably. Further, concerns regarding contamination of marine sources with mercury and organic pollutants, taste, smell and non-vegetarian nature of fish have constrained their utilization. Recently, plant-based PUFA resources are gaining much importance because of their sustainable supply, safety and dietary acceptance$^5$. $\alpha$-linolenic acid (ALA; 18:3 n-3) is the major terminal $\omega$-3 fatty acid found in most commonly available PUFA rich plant seeds and their oils, specifically flax, chia, soybean, hemp, canola and walnuts$^6$. However, consumption of ALA-rich plant oils doesn’t confer the similar health benefits of EPA and DHA as the efficiency of conversion of ALA to EPA and DHA in humans is limited due to rate limiting step catalysed by hepatic enzyme Delta-6 desaturase (D6D)$^6$. Hence, to meet increased demand for PUFA rich oils, many food and nutraceutical industries and research organisations are interested in genetically improving con-
ventitional oilseed crops or agronomically adapting selected plant species rich in unique ω-3 fatty acids such as SDA. *Buglossoides arvensis* (L.) M. Johnston (Field Gromwell or Corn Gromwell), is an annual herbaceous weed of Boraginaceae family. The plant is native to northern temperate regions of Asia and Europe. The plant produces warty seeds, which are trigonous-conical in shape and greyish-brown in color. This unique and underutilized oilseed plant has been recently exploited for its high PUFA content and also for its natural ability to synthesize and accumulate an unusual ω-3 fatty acid, namely Stearidonic acid (SDA; 18:4 n-3) in its seeds. Further, *B. arvensis* seed oil is superior in terms of SDA content to other plant sources of SDA such as black currant (2-4% SDA) and echium (12-14% SDA). In addition to SDA, the seeds also contain therapeutically important fatty acids such as γ-linolenic acid (GLA; 18:3 n-6) and ALA (18:3 n-3). Mounting research evidence from supplementation studies and randomized clinical trials in humans and animal models indicate that dietary consumption of plant oils rich in SDA increase the tissue EPA levels more efficiently (2.2-4 times) than plant oils rich in ALA because it bypasses the rate-limiting step catalyzed by D6D. Further, dietary consumption of *B. arvensis* seed oil has been reported to increase the circulating n-3 PUFA levels in a dose-dependent manner and associated with anti-inflammatory phenotype in healthy subjects.

*B. arvensis* is yet to be agronomically adapted and commercially cultivated worldwide. Currently, high yielding cultivars of *B. arvensis* are developed, commercially grown only in the UK and the oil is marketed in the United States and European Union under the trade name Ahiflower oil. In India, *B. arvensis* grows as a weed species at higher elevations in the northern temperate (cold climatic conditions) regions of India (Pampore, Jammu and Kashmir; Latitude 34.02° N and longitude 74.93° E). Our group collected the *B. arvensis* mature seeds from the above native habitat, successfully adapted and also assessed their suitability for large-scale commercial cultivation in tropical regions (hot climatic conditions) of south India.

The oil content of *B. arvensis* produced in Spain and other regions of Europe are reported to vary from 10-21%. Likewise, the content of PUFAs such as SDA, GLA and ALA in *B. arvensis* seeds was also reported to vary in the ranges of 14-21%, 4-8% and 39-48%, respectively. In general, the oil content and fatty acid composition of oilseeds vary with various factors such as geographical locations, agricultural practice, climatic condition and cultivar or the variety of the plant. Cumberford et al. reported the fatty acid composition and quality parameters of *B. arvensis* seed oil extracted from UK cultivars. To the best of our knowledge, there is no documented literature available on fatty acid composition and physico-chemical characteristics of Indian *B. arvensis* seed oil. Further, molecular characterization of major constituents of *B. arvensis* seed oil such as lipid classes and triacylglycerol (TAG) provides important information on the behavior of lipids during processing and are not yet reported in any literature. Hence, the present study was carried out to investigate the oil content, physicochemical characteristics, fatty acid composition of total lipids and lipid classes, tocopherol content and triacylglycerol (TAG) molecular species of the oil extracted from the high yielding cultivar of *B. arvensis* (BA-26), cultivated in tropical regions of south India. The current study also aimed to determine FT-IR spectroscopic profile and the oxidative stability of *B. arvensis* seed oil in comparison to other PUFA and MUFA rich oils. We strongly anticipate that the characterization data will assist industries and research groups for future research on the preparation of value-added products using this nutritionally superior SDA rich seed oil.

2 Materials and methods

2.1 Plant material and growth conditions

Adaptation, selection and evaluation of high yielding cultivar of *B. arvensis* (BA-26) were reported in our previous study. Mature seeds of BA-26 cultivar were subject to germination by exposing them to cold stratification (4-6°C) for 4-7 days. Germinated seeds were sown and plants were cultivated with protective irrigation in open field beds (2 m x 3 m) located at CSIR-Central Food Technological Research Institute research farm, Mysuru, India (red loamy soil; longitude 76°37’ 16.1364°E; latitude 12°19’ 39.3456”N; average altitude 770 MSL and average rainfall 804.2 mm). Mature seeds were harvested at the end of crop cycle (130-140 days). The seeds from three generations were used in the present study. The moisture content of the seeds was determined according to ISTA standard procedure.

2.2 Chemicals and standards

Fatty acid methyl ester mix (F.A.M.E. mix, C4-C24), Stearidonic acid, α-tocopherol, β-tocopherol, γ-tocopherol, and porcine pancreas lipase were purchased from Sigma-Aldrich (Saint Louis, MO, USA). All solvents and chemicals used in the present study were of analytical grade obtained from Merck, USA and Himedia, India.

2.3 Extraction of seed oil

Oil extraction was carried out using a Soxhlet apparatus fitted with a condenser and a 1 L round bottom flask. Briefly, *B. arvensis* seeds collected from native habit (Pampore, Jammu and Kashmir) and BA-26 cultivar were crushed into a fine powder using mortar and pestle. About 100 grams of ground powder was fed into soxhlet apparatus and extraction was carried out using hexane as a solvent for about 6 h. The solvent was removed under
vacuum using a rotary evaporator. Recovered oil was collected in an amber color bottle, flushed with a stream of nitrogen gas and stored at 4°C until use. Oil content was determined on dry weight basis.

2.4 Fatty acid profiling of *B. arvensis* seed oil

The fatty acid composition of *B. arvensis* seed oil extracted from wild species and *BA*-26 cultivar were determined by using gas chromatography-mass spectrophotometer (GC-MS). The Fatty acid methyl esters were prepared by esterification oil with 1 mL of BF₃-methanol complex solution (13-15% BF₃ basis Sigma, USA) at 65°C for 1 hr. After cooling in ice for 5min, FAMEs were extracted by adding water: hexane (1:1). Tubes were vortexed and centrifuged at 1000 g for 5 min. The upper hexane layer containing FAMEs was transferred to GC vial, dried under the nitrogen stream and then re-suspended in MS grade hexane. Samples were analysed using Agilent DB-23 column (50% Cyanopropyl)–methylpolysiloxane; 60 m length; 0.25 mm ID; 0.25 μm film thickness) on 7890B GC-5977A MSD system (M/s Agilent technologies, Singapore). GC conditions performed as follows: Helium at a flow rate with 3 min hold. Mass spectra were recorded under EI at a fixed electron energy of 70 eV. Fatty acids identified by comparing their mass spectrum to authentic standards and the results were confirmed by a mass spectral library search (NIST version 2.0 g). All the experiments were performed in biological triplicates and the results are expressed as mean ± SD.

2.5 Physicochemical analysis

Colour of the extracted seed oil was determined using Lovibond Tintometer (Model-F, The Tintometer Ltd., Salisbury, UK). Refractive index (RI) was calculated at 30°C using Abbe Refractometer (Model NAR-3 T, ATAGO Co., Ltd., Tokyo, Japan). Specific gravity (SG) was measured at 25°C using 10mL Pycnometer (Borosil, India). Viscosity was measured at 25°C and 40°C using Brookfield viscometer (Model DV-III, Stoughton, MA, USA). The moisture content of the oil was determined using a hot air oven method.

Peroxide value (PV), Saponification value (SV) and Iodine value (IV) were determined according to American Oil Chemists’ Society (AOCS) official methods 965.33, 920.160 and 920.159 respectively. The α-Anisidine value determined according to AOCS official method Cd 18-90. Acid value (AV) and free fatty acid (FFA) were determined according to ISO standard method.

2.6 Quantification of tocopherols

Tocopherols were separated and quantified by using HPLC. Oil (1 g) was saponified with 1 mL of KOH, 4 mL of 5 % ethanolic pyrogalol (w/v) and boiled for 3 min in a water bath. The reaction was stopped by cooling in ice for 5 min followed by addition of 30 mL of distilled water. Tocopherols were extracted thrice with diethyl ether, washed with water and dried under vacuum at 40°C. The residue was re-suspended in 4 mL of benzene and 1 mL of ethanol and dried under a stream of nitrogen. The residue was finally dissolved in ethanol for analysis by HPLC (Nexera X-2 LC-30A, Kyoto, Japan) equipped with an LC-30AD solvent delivery pump, SIL-30AC auto-sampler, DGU-20A5R degassing unit, RF-20A fluorescence detector, Phenomenex C18 column (250 × 4.60 mm, 5 μm) and LabStation software. Mobile phase consisted of methanol: water (95:5, v/v). Excitation and emission wavelengths were set at 290 nm and 330 nm, respectively. The flow rate was 1 mL/min. Tocopherol isomers were identified by comparing their retention time to known standards (α, β, γ- tocopherols, Sigma, USA) and quantified by an external standard method. All estimations were performed in triplicate and results were expressed as mean ± SE.

2.7 Determination of oxidative stability

Oxidative stability of the oil was determined by using Oxitest method (AOCS international standard procedure Cd 12c-16). Briefly, 5 g of oil was transferred to oxidation chambers of OXITEST reactor (VELP Scientifica, Usmate, Milan, Italy). Oil was subjected to accelerated oxidation at 90°C with an oxygen pressure of 6 bars. Induction period (IP) was calculated from oxidation curves using least square method (LSM) in OXISoft software program (VELP Scientifica). Oxidative stability of sunflower oil was also evaluated for comparison using the same conditions.

2.8 Fractionation of lipid classes by column chromatography and determination of their fatty acid composition

Oil was fractionated into neutral lipids (NL), glycolipids (GL) and phospholipids (PL) using silica gel column chromatography. Briefly, a glass column (Borosil; 30 cm × 20 mm i.d.) was packed with the slurry of activated silica gel (30 g; 100-200 mesh; Merck) in chloroform (1:5). Oil (30 mg/1g adsorbent) was dissolved in 10 mL of chloroform and applied to the column. NL, GL and PL were sequentially eluted from the column using the solvents chloroform, acetone and methanol respectively. Solvent from each lipid class was evaporated under vacuum using a rotary evaporator and the amount of each lipid classes was determined gravimetrically. NL GL and PL fractions were further

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screened for identification of components by thin-layer chromatography using the solvent systems, petroleum ether: diethyl ether: acetic acid (70:30:1), chloroform: methanol: water: acetic acid and chloroform: methanol: water (65:25:4) respectively. Each lipid class was dissolved in respective eluting solvents and stored at −20°C until further analysis. The fatty acid composition of each lipid classes was determined by GC-MS.

2.9 Identification of triacylglycerol molecular species by high resolution mass spectrometry (HRMS)

Triacylglycerols (TAGs) were separated from the oil according to AOAC official method cd 11c-93\textsuperscript{10} using silica gel column chromatography. About 0.9 g of oil dissolved in 5 mL of chloroform was added to the glass column (Borosil; 30 cm × 20 mm i.d.) packed with a slurry of activated silica gel in petroleum ether. The TAG was eluted using the solvent system comprising petroleum ether: diethyl ether (225:25, v/v). Solvent from TAG fraction was evaporated under vacuum using rotary evaporator. Purified TAG fraction was confirmed by TLC and fatty acid composition was determined by GC-MS.

About 750 μg of the purified TAG was mixed with 15 μg of internal standard (tri17:0-TAG) and dissolved in 1 mL of Chloroform: methanol (1:2) plus 10 mM ammonium acetate. The samples were introduced by direct infusion at a flow rate of 7 μL/min into ESI source (DuoSpray\textsuperscript{®} ion source) on a TripleTOF\textsuperscript{™} 5600 system (AB SCIEX Concord, ON, Canada) controlled with Analyst\textsuperscript{®} TF 1.6 software with MS/MS\textsuperscript{ALL} mode activated. The molecular species of the TAG was analysed in positive mode. In MS/MS\textsuperscript{ALL} mode, TOF MS experiment was performed from m/z 100-1200 at an accumulation time of 300 ms, followed by 1100 product ion scan as described earlier\textsuperscript{10}. TOF MS experiment parameters include ion source gas 1 at 15 psi, ion source gas 2 at 20 psi, curtain gas at 25 psi, ESI source temperature of 200°C, ion spray voltage at +5100V (positive ion mode) and accumulation time of 3000 ms. Collision energy (CE) of +10 eV and de-clustering potential (DP) of +80 eV were used. Product ion experiment parameters include similar gas/sources values as described for TOF MS experiment but with an accumulation time of 300 ms. CE of +50 eV and DP of +80 eV were used. The instrument was calibrated using AB SCIEX mode specific calibration solutions. A wash step and sample blanks were included between each sample to assess the carryover.

The acquired data were processed with Peak View 2.2 software (AB SCIEX). Background spectrum of each sample was subtracted using blank spectra. TAG molecular species were identified as [M + NH\textsubscript{4}]\textsuperscript{+} ions by a series of neutral loss (NL) scans as described by earlier\textsuperscript{20}. Loss of fatty acyl chains as neutral ammoniated fragments were targeted in NL scans; NL283 (17:0); NL273 (16:0); NL293 (18:4); NL295 (18:3); NL297 (18:2); NL299 (18:1); NL301 (18:0); NL323 (20:3); NL325 (20:2); NL327 (20:1); NL329 (20:0); NL355 (22:1); NL357 (22:0). At each m/z, TAG molecular species were annotated by their total fatty acyl carbons: total fatty acyl double bonds (e.g. 54:9). The combination of acyl chains of TAGs at a particular m/z was extracted by fragmentation analysis and product ion scanning in Peak View software. Relative intensity was obtained from MS peak area. All the experiments were done in triplicate and the results are expressed as Mean ± SD.

2.10 Fatty acid composition and positional distribution of triacylglycerol

Fatty acid positional distribution of B. arvensis TAGs was analysed according to the method described by earlier\textsuperscript{21}. Briefly, 25 mg of the purified TAG added to 5 mL of 1M Tris-HCl buffer (pH: 8) containing 1.25 mL of 0.05% sodium taurocholate and 0.5 mL of 2.2% calcium chloride. About 5 mg of porcine pancreatic lipase was added to the reaction mixture and incubated in a shaking water bath at 40°C for 15 min under a nitrogen stream. The reaction was stopped by adding 5 mL of ethanol and 5 mL of 6M HCl. The hydrolytic products were extracted using hexane, washed twice with distilled water, filtered through a bed of sodium sulphate, evaporated to dryness under the nitrogen stream and separated on the silica-TLC plate using the solvent system-petroleum ether: diethyl ether: acetic acid (1:2)لا она خليط. The reaction mixture was incubated in a shaking water bath at 40°C for 15 min under a nitrogen stream. The reaction was stopped by adding 5 mL of ethanol and 5 mL of 6M HCl. The hydrolytic products were extracted using hexane, washed twice with distilled water, filtered through a bed of sodium sulphate, evaporated to dryness under the nitrogen stream and separated on the silica-TLC plate using the solvent system-petroleum ether: diethyl ether: acetic acid (1:2)لاق. The reaction mixture was incubated in a shaking water bath at 40°C for 15 min under a nitrogen stream.

2.11 Acquiring Fourier Transform Infrared (FTIR) spectra

Tensor II FTIR spectrometer (Bruker, Germany) coupled with a deuterated triglycine sulphate (DTGS) detector and platinum ATR accessory was used to acquire FTIR spectra of the oil. Brieﬂy, a small droplet of oil was placed on the flat sensing surface of the diamond ATR crystal and the spectra were recorded at a resolution of 4 cm\textsuperscript{-1} from 4000 to 5000 cm\textsuperscript{-1} using 64 co-added scans. A spectrum of a clean ATR surface was used as background. FTIR bands were assigned to the specific functional groups according to previous FTIR studies on PUFA rich oils\textsuperscript{22–26}.

Statistical analysis

All the experiments were performed in triplicate and the results were expressed as Mean ± SD. Statistical analysis was performed using SPSS statistical software version 20 (IBM Corporation, NY, USA).
3 Results and discussion

3.1 Oil content and fatty acid composition

Any oilseed crop with an oil yield of >15% (w/w of dry mass) is considered as economically viable raw material for edible oil production\(^{31}\). The oil yield of *B. arvensis* seed oil extracted from the BA-26 cultivar (grown in the tropical climate) was found to be 18.5% (dry weight basis), making it an ideal candidate for commercial exploitation. Oil content of BA-26 is almost comparable to the oil content (19.3%) of wild species from India (grown in temperate climate) and UK cultivars (19-21%)\(^3\) but higher than the oil yield reported earlier\(^9\) for *B. arvensis* from Spain (10.72%) (Table 1). The fatty acid composition of *B. arvensis* seed oil (BSO) extracted from BA-26 cultivar and wild species from India along with fatty acid compositions of BSO from different origins reported in previous studies\(^3,9\) are presented in Table 1. Representative GC-MS chromatogram of BSO along with lipid profile of the oil is presented in Fig. 1. The results of the fatty acid composition of BSO from BA-26 cultivar is comparable to those for the wild species from India, indicating that the cultivation of *B. arvensis* plant in tropical climatic conditions didn’t show much variation. Both showed PUFA such as linoleic acid, \(\alpha\)-linolenic acid and stearidonic acid as dominant fatty acids followed by MUFA such as oleic acid (OA) and saturated fatty acids (palmitic and stearic acid). However, it is evident from Table 1 that variation in the composition of fatty acids among different geographical location exists, specifically with regard to PUFA such as GLA, SDA and ALA. The reported value of SDA content in BSO from Spain\(^9\) was 14.08% of total fatty acids, which is significantly low compared to those from the UK cultivars (20% SDA)\(^3\), BA-26 cultivar (18.6% SDA), and wild species from India (19.5% SDA). ALA content of BSO from BA-26 cultivar and wild species from Indian was found to be 47.5% and 45% respectively, which was high compared to those reported for BSO from the Spain (39.68%) and the UK cultivars (41.5%). The content of GLA was found to be less in BA-26 cultivar (3.9%) and wild species from India (4.4%) compared to those from the UK (6.0%) and Spain (6.44%)\(^3,9\), which may be mainly due to high levels of ALA observed in the oil from Indian species. The observed variation in the oil content and fatty acid composition among *B. arvensis* from different regions may be attributed to various factors such as the nature of the seed, geographic location, growing practices and genetic factors.

Total fatty acid profile of BSO from BA-26 cultivar is distinctive as it contains 81.3% polyunsaturated fatty acids comprising 66.1% of \(\omega-3\) fatty acids and 15.2% of \(\omega-6\) fatty acids (Table 1). Unsaturated to saturated fatty acid ratio and the \(\omega-6/\omega-3\) fatty acid ratio of BSO was found to be 9.75 and 0.22, respectively and was comparable to those

| Fatty acid                 | Relative percentage (%) of total fatty acids |
|---------------------------|---------------------------------------------|
|                           | B. arvensis seed oil from BA-26 line         | B. arvensis seed oil from wild species       | B. arvensis seed oil from Spain\(^a\) | Ahiflower oil (Europe)\(^b, c\) |
| Oil content (% of total lipids) | 18.5 ± 0.4                                   | 19.3 ± 0.5                                   | 10.72                                    | 19-21%                             |
| Palmitic acid (C16:0)      | 6.7 ± 0.2                                    | 7 ± 0.18                                     | 9.41                                     | 4.5                                 |
| Stearic acid (C18:0)       | 2.6 ± 0.19                                   | 3.6 ± 0.2                                    | 2.81                                     | 1.8                                 |
| Oleic acid (C18:1 n-9)     | 9.4 ± 0.6                                    | 10.9 ± 0.2                                   | 6.83                                     | 8.5                                 |
| Linoleic acid (C18:2 n-6)  | 11.3 ± 0.4                                   | 9.6 ± 0.1                                    | 14.8                                     | 12.5                                |
| \(\gamma\)-linolenic acid (C18:3 n-6) | 3.9 ± 0.05                                    | 4.4 ± 0.09                                   | 6.44                                     | 6.0                                 |
| \(\alpha\)-linolenic acid (C18:3 n-3) | 47.5 ± 0.35                                    | 45 ± 0.8                                     | 39.68                                    | 41.5                                |
| Stearidonic acid (C18:4 n-3) | 18.6 ± 0.7                                   | 19.5 ± 0.1                                   | 14.08                                    | 20.0                                |
| Total SFAs                | 9.3                                         | 10.6                                         | –                                        | –                                   |
| Total MUFA                | 9.4                                         | 10.9                                         | –                                        | –                                   |
| Total PUFA                | 81.3                                        | 78.5                                         | –                                        | –                                   |
| U/S ratio                 | 9.75                                        | 8.43                                         | –                                        | –                                   |
| \(\omega-6/\omega-3\) ratio | 0.22                                        | 0.21                                         | –                                        | –                                   |

All the experiments were done in triplicate and the results are expressed as Mean ± SD.

\(^a\) Guí-Guerrero, 2001  
\(^b\) Cumberford & Hebard, 2015  
\(^c\) Surette, 2013
Fig. 1  Thin layer chromatography (TLC) separation and GC-MS chromatogram of *B. arvensis* seed oil. (A) TLC profile of BSO. Seed oil was separated on silica TLC plate using the solvent system, petroleum ether:diethyl ether:acetic acid (70:30:1). TLC plate was developed by manganese chloride charring. (B) GC-MS chromatogram showing fatty acid profile of BSO. (C) Mass spectrum of stearidonic acid (SDA; C18:4). DAG, diacylglycerol; FFA, free fatty acid; TAG, triacylglycerol; SE, steryl ester; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3 (n-6), γ-linolenic acid; C18:3 (n-3), α-linolenic acid.

Table 2  Physico-chemical characteristics, content of lipid classes and tocopherols of *B. arvensis* seed oil.

| Parameter                        | Value               |
|----------------------------------|---------------------|
| **Physical parameters**          |                     |
| Colour                           | 0.3R, 9.1Y          |
| Refractive index at 30°C         | 1.48 ± 0.03         |
| Viscosity at 25°C                | 45.5 ± 0.4          |
| Specific gravity at 25°C (mPa.s)| 0.933 ± 0.01        |
| Physical state at 4°C            | Liquid              |
| **Chemical parameters**          |                     |
| Free fatty acid (% of oleic acid)| 1.28 ± 0.25         |
| Acid value (mg of KOH/g of oil)  | 2.55 ± 0.51         |
| Saponification value (mg of KOH/g of oil) | 195 ± 5.7 |
| Iodine value (g of I2/100g of oil)| 217.2 ± 5.5       |
| Peroxide value (meq of active oxygen/kg of oil) | 2.06 ± 0.09 |
| p-anisidine value                | 10.7 ± 0.29         |
| Induction period (IP; oxidative stability) | 3.1 hours |
| **Tocopherols Content (mg/kg of oil)** |                     |
| α-Tocopherol                     | ND                  |
| β-Tocopherol                     | 32.84 ± 1.7         |
| γ-Tocopherol                     | 741.64 ± 2.6        |
| Total tocopherols                | 774.8 ± 0.9         |

All the experiments were conducted in triplicate and the results are expressed in Mean ± SD.

for wild species from India (8.43 and 0.21, respectively) (Table 1). Many global health organizations have recommended intake of ω-6/ω-3 fatty acids ratio of 2:1 or lower for reducing the risk of various chronic diseases including cardiovascular disease, inflammation and autoimmune diseases. Overall, the presence of good amounts of nutritionally important PUFAs such as SDA, ALA and GLA, makes the Indian *B. arvensis* seed oil a special ingredient for various nutritional applications.

3.2 Physicochemical characterization

In the current study, oil extracted from *B. arvensis* seeds (BA-26) was evaluated for its quality attributes. The results of physico-chemical characteristics are presented in Table 2. Moisture content is an important parameter that determines the quality of oils. Vegetable oils with high moisture content are more prone to hydrolytic rancidity. The moisture content of BSO was found to be 0.14-0.25 g/100 g of oil, which is within limits reported for most of the vegetable oils. The color of the extracted seed oil was greenish-yellow and showed 0.3 red units and 9.1 yellow units in Lovibond tintometer. The viscosity of BSO was found to be 45.5 mPa.s at 25°C and decreased to 43.4 mPa.s at 40°C, suggesting that BSO has a low amount of saturated fat. Refractive index (RI) and specific gravity (SG) are important parameters used to determine the authenticity and composition of oils. RI of BSO was 1.48, which is higher than MUFA rich oils such as sunflower (1.46) and olive oil (1.47) but comparable to other PUFA rich vegeta-
bable oils like chia (1.48) and flax seed (1.48)\textsuperscript{13}. This is due to the fact that RI increases with increasing degree of unsaturation and fatty acid chain length. The SG of oils is used to assess the changes in the degree of saturation and composition during processing. The SG of BSO was found to be 0.933 at 25°C, which is higher than sunflower oil (0.92) and olive oil (0.92) but comparable to chia and flax seed oil (0.93)\textsuperscript{13}.

Free fatty acid (FFA) and acid value (AV) are the key parameters that affect the commercial value and quality of the edible oils. FFAs are produced by oil hydrolysis during extraction, processing and storage. They are less stable and more prone to oxidation, making the oil rancid\textsuperscript{29}. FFA and AV of BSO extracted in this study were found to be 1.28 (\% of oleic acid) and 2.55, which is higher than those reported for refined BSO from UK (AV = 0.12 to 0.34) and other edible vegetable oils like sunflower and flax seed oil suggesting that BSO requires refining to make it suitable for the edible purpose. Further, utmost care must be taken to prevent hydrolytic reactions in the oil during extraction. Saponification value (SV), an index of nature of fatty acids present in the oil was found to be 195 mg of KOH/g of oil. SV of BSO is slightly higher than that of other vegetable oil such as sunflower oil (188-194 mg of KOH/g of oil), soybean oil (189-195 mg of KOH/g of oil)\textsuperscript{29}, indicating that BSO has a high content of short-chain fatty acids than oils mentioned above. Iodine value (index of unsaturation levels) was found to be 217.2 g of Iodine per 100 g of oil, which is higher than other PUFA and MUFA rich vegetable oils like chia seed oil (204), linseed oil (187), sunflower oil (118-141), safflower oil (136-148) and olive oil (75-95)\textsuperscript{13, 30}. This is attributed to the relatively higher content of PUFA in BSO, particularly SDA, LA, ALA and GLA. Oils rich PUFA are more susceptible to oxidative damage. PV and p-anisidine values are the two key parameters used to determine the primary and secondary oxidation state of the edible oils respectively\textsuperscript{17}. The PV of BSO was 2.06 meq of active oxygen/kg of oil, which is far below the maximum recommended limit of PV for edible oils by Codex Alimentarius (PV: 10 meq of active oxygen/kg of oil) and comparable to those reported for refined BSO from the UK. p-anisidine and total oxidation value (TOTOX) value (p-anisidine value + 2 PV) of BSO was found to be 10.7 and 14.82 respectively, which is far below the maximum recommended limit (p-anisidine value: 20; TOTOX: 26) by Global Organization for EPA and DHA Omega-3s (GOED)\textsuperscript{31}. Low values of PV, p-anisidine and TOTOX of BSO extracted in the current study indicate that no detrimental oxidation reaction occurred during the extraction and processing of the oil. However, oils rich in PUFA are more prone to oxidative degradation during long-term storage and hence need to be protected to extend the shelf-life either by addition of antioxidants or by soft gel encapsulation\textsuperscript{13}.

3.3 Tocopherols content

Tocopherols are the lipid soluble compounds found in minor quantities in the seed oils. They are natural antioxidants and have free radical scavenging ability involving tocopherol-tocopheryl semiquinone redox system\textsuperscript{32}. γ-tocopherol (741.64 mg/kg of oil) is the major tocopherol found in the B. arvensis seed oil followed by β-tocopherol (32.84 mg/kg of the oil) (Table 2). Other vegetable oils such as rape seed oil, hemp oil and sacha inchi seed oil also have γ-tocopherols as a predominant tocopherol isomer\textsuperscript{32, 33}. Earlier reports showed that the antioxidant capacity of tocopherol isomers decreases in the order of γ>δ>β>α-tocopherol\textsuperscript{13}. Hence, a large amounts of γ-tocopherols found in the BSO, make it less susceptible to oxidation.

3.4 Oxidative stability

Oxidative stability studies using OXITEST provides an estimate of susceptibility of oils to oxidation. Accelerated oxidation test (OXITEST) showed that induction period (IP) of BSO was found to be 3.1 h, which is lower than to sunflower oil (6 h) (Table 2). Low oxidative stability of BSO is due to the high unsaturated fatty acid levels in the oil compared to sunflower. Hence, utmost care should be taken to prevent oxidation of BSO during extraction, processing, storage and transportation.

3.5 Lipid classes of BSO and their fatty acid composition

Estimation of lipid classes is of great importance as they affect the quality of the oil because of their different fatty acid composition. Few studies reported that identification and characterization of lipid classes specifically polar lipids might provide valuable information on molecular fingerprint for authenticity purpose and to determine adulteration in oils\textsuperscript{34}. Lipid class of BSO was separated by using silica column chromatography. Neutral lipids (89.3\%) were found to be major lipid classes followed by glycolipids (7.4\%) and phospholipids (3.3\%) (Table 3).

The content of fatty acids in neutral lipids differs significantly from glycolipid and phospholipid. ALA (43.4\%) and SDA (16.5\%) were the dominant fatty acids in the neutral lipid fraction whereas ALA (29.9\%) and palmitic acid (PA; 18.3\%) were the main fatty acids in glycolipids. Phospholipids have more SFAs such as PA (44.8\%) and stearic acid (SA; 12.9\%) whereas the content of PUFAs such as ALA and SDA accounts for 12.5\% and 2.9\%, respectively. GLA content of neutral lipids was high (5.5\%) compare to glycolipids (4.1\%) and phospholipids (2.6\%). Neutral lipids, a major lipid class comprising a large portion of BSO was found to have high PUFA content (78.8\%) compare to glycolipids (58.1\%) and phospholipids (30.8\%). Further, unsaturated to the saturated fatty acid ratio of neutral lipid (8.9) was high compared to glycolipid (2.7) and phospholipids (0.7), making the oil PUFA rich.
3.6 Identification of triacylglycerol molecular species by high resolution mass spectrometry (HRMS)

Plant seeds store TAG as sources of structural fatty acids and energy to the developing embryo. They are the major neutral lipids found in all vegetable oils including BSO and provide ~25% of dietary calories in developed countries\(^{20}\). BSO TAG was purified using silica column chromatography and its fatty acid composition analyzed by GC-MS is presented in Table 4. ALA (47.9 %) and SDA (16.7 %) were the predominant fatty acids found in the BSO triacylglycerols followed by LA (12.4 %), OA (10.2 %), PA (5.9 %), GLA (4.3 %) and SA (2.2 %). The unsaturated fatty acid content of BSO TAGs was found to be 91.5 % whereas saturated fatty acid accounts for only 8.5 %. The fatty acid composition of triacylglycerols is used to evaluate the nutritional quality and stability of oils. However, to understand the physico-chemical properties and lipid behavior during processing, determination of the type of TAG molecular species in the oil is essential\(^{11,33}\).

Vegetable oils have a complex mixture of triacylglycerol molecular species and are composed of glycerol backbone esterified with unsaturated, saturated fatty acyl chains or a mixture of both. TAG molecular species of BSO was analysed by direct infusion of the purified TAG into HRMS through ESI source in positive mode. A spectrum of ammoniated TAGs shows five clusters of MS peaks that represents C52, C54, C56, C58 and C60 (Fig. 2A). Ammonium adducts of

### Table 3
Lipid classes content and the fatty acid composition of *B. arvensis* seed oil.

| Fatty acid                | Relative % of total fatty acids |
|---------------------------|---------------------------------|
|                           | Neutral lipid | Glycolipid | Phospholipid |
| **Content**               |               |            |              |
| (% of dry weight)         |               |            |              |
| Palmitic acid (PA, C16:0) | 7.2 ± 0.64    | 18.3 ± 2.09| 44.8 ± 2.24  |
| Stearic acid (SA, C18:0)  | 2.9 ± 0.56    | 8.7 ± 1.19 | 12.9 ± 0.23  |
| Oleic acid (OA, C18:1 n-9)| 11.1 ± 0.82   | 14.9 ± 0.73| 11.5 ± 0.28  |
| Linoleic acid (LA, C18:2 n-6) | 13.4 ± 0.49  | 15.2 ± 0.85| 12.8 ± 0.49  |
| γ-linolenic acid (GLA, C18:3 n-6) | 5.5 ± 0.09 | 4.1 ± 0.69 | 2.6 ± 0.06   |
| α-linolenic acid (ALA C18:3 n-3) | 43.4 ± 2.13 | 29.9 ± 2.07| 12.5 ± 1.1   |
| Stearidonic acid (SDA, C18:4 n-3) | 16.5 ± 0.33 | 8.9 ± 1.19 | 2.9 ± 0.34   |
| **Total SFAs**            | 10.1          | 27         | 57.7         |
| **Total MUFA**            | 11.1          | 14.9       | 11.5         |
| **Total PUFA**            | 78.8          | 58.1       | 30.8         |
| **U/S ratio**             | 8.9           | 2.7        | 0.7          |
| **ω-6/ω-3 ratio**         | 0.31          | 0.49       | 1            |

All the experiments were conducted in triplicate and the results are expressed in Mean ± SD.

### Table 4
Fatty acid composition and positional distribution of triacylglycerol.

| Fatty acids                | % of Total fatty acids |
|---------------------------|------------------------|
|                           | TAG | sn-2   | sn-1, 3 |
| Palmitic acid (16:0)      | 5.9 ± 0.11 | 1.9 ± 0.6 | 11.5 ± 1.1 |
| Stearic acid (18:0)       | 2.2 ± 0.14 | 4.2 ± 1.6 | 5.3 ± 0.4  |
| Oleic acid (18:1)         | 10.2 ± 0.18 | 16 ± 0.3 | 12.8 ± 0.7 |
| Linoleic acid (18:2)      | 12.4 ± 0.28 | 17.1 ± 0.25 | 12 ± 0.02 |
| γ-linolenic acid (18:3)   | 4.3 ± 0.25 | 7 ± 0.5 | 1.1 ± 0.14 |
| α-linolenic acid (18:3)   | 47.9 ± 0.84 | 32.4 ± 1.5 | 51.9 ± 2.1 |
| Stearidonic acid (18:4)   | 16.7 ± 0.21 | 21.4 ± 0.5 | 5.4 ± 0.46 |

All the experiments were conducted in triplicate and the results are expressed in Mean ± SD.
TAG[^M + NH₄]^+ were identified through a series of neutral loss scans: NL273 (16:0); NL293 (18:4); NL295 (18:3); NL297 (18:2); NL299 (18:1); NL301 (18:0); NL323 (20:3); NL325 (20:2); NL327 (20:1); NL329 (20:0); NL355 (22:1); NL357 (22:0) (Fig. 2B). In the current study, TAG molecular species were represented by their total carbon number and number of double bonds in their three fatty acyl chains, by their fatty acyl combination and by their mass (m/z). The combination of fatty acyl chains of TAGs was deduced by fragmentation analysis and product ion scanning. Neutral loss of fatty acids from the glycerol backbone of intact TAG yields product ions of diacylglycerol. Hence, one TAG mass (m/z) can be resolved into triacylglycerols with multiple fatty acyl combinations. For example, ammonium adduct of the TAG at m/z 890.8 represents TAG molecule(s) with 54 carbons and 9 double bonds in the
of ammonium adduct of TAG \[ \text{NH}_3 \], 18:3

m/z 597.6 corresponding to the NL or dissociation of fatty acids 18:2, 18:3 and 18:4, respectively.

(B) The theoretical m/z of C54:8 (Singly charged ammoniated ion) is 892.2. Fragmentation of precursor ion at m/z 892.8 generates DAG product ions at m/z 593.6, 595.6 and 597.6, which indicates the neutral loss of fatty acids 18:1, 18:2, 18:3 and 18:4, respectively. Fatty acyl combinations at each m/z were represented above.

Fig. 3

Product ion scanning of TAG molecular species at m/z 890.8 and 892.8 (A) the theoretical m/z of C54:9 (Singly charged ammoniated ion) is 890.2. Fragmentation of precursor ion at m/z 890.8 generates DAG product ions at m/z 593.6, 595.6 and 597.6, which indicates the neutral loss of fatty acids 18:2, 18:3 and 18:4, respectively. (B) The theoretical m/z of C54:8 (Singly charged ammoniated ion) is 892.2. Fragmentation of precursor ion at m/z 892.8 generates DAG product ions at m/z 593.6, 595.6, 597.6 and 599.6, which indicates the neutral loss of fatty acids 18:1, 18:2, 18:3 and 18:4, respectively. Fatty acyl combinations at each m/z were represented above.

three fatty acyl chains. Fragmentation of m/z 890.8 yielded three diacylglycerol product ions at m/z 593.6, 595.6 and 597.6 corresponding to the NL or dissociation of fatty acids 18:2 + NH\textsubscript{3}, 18:3 + NH\textsubscript{3} and 18:4 + NH\textsubscript{3}, respectively, indicating that C54:9-TAG at m/z 890.8 have four TAG molecular species with different acyl combination namely, 18:4-18:3-18:2 and 18:3-18:3-18:3 (Fig. 3A). Similarly, fragmentation of ammonium adduct of TAG (54:8) at m/z 892.8 yielded four DAG product ions at m/z 593.6, 595.6, 597.6 and 599.6 corresponding to the NL of fatty acids 18:1 + NH\textsubscript{3}, 18:2 + NH\textsubscript{3}, 18:3 + NH\textsubscript{3} and 18:4 + NH\textsubscript{3}, representing three TAG molecular species namely, 18:4-18:3-18:1, 18:3-18:3-18:2, 18:4-18:2-18:2 (Fig. 3B). Overall, 37 TAG masses (m/z) representing 161 TAG molecular species were identified through the spectra of eleven NL scans followed by fragmentation analysis and product ion scanning. Predominant triacylglycerol molecular species were detected at m/z 888.8 (C54:10), 890.8 (C54:9), 892.8 (C54:8), 894.8 (C54:7), 896.8 (C54:6) and 898.8 (C54:5). Out of 161 TAG molecular species, 40 TAGs were identified to have SDA. Relative intensity at each TAG mass was calculated as the ratio of MS peak area with respect to the sum of MS peak areas of all identified TAGs (Table 5). At molecular level, ω-3 fatty acids such as SDA and ALA were identified on TAG backbone with higher levels of mono and di-unsaturated fatty acids (18:1, 18:2, 20:1, 20:2, 22:1) than saturated fatty acids (16:0, 18:0, 20:0, 22:0). This will largely affect various physicochemical properties of the oil specifically, the fluidity of oil at 4°C and RI, SG and IV. Due to the complexity of the BSO TAGs, sn-position of fatty acyl chains on glycerol backbone were not characterized using HRMS. Overall, the TAG molecular characterization data provides in this study can be utilized to understand the behavior of lipids during B. arvensis oil processing.

3.7 Fatty acid profiling and regio-distribution of BSO triacylglycerols

TAGs found in the vegetable oils are large diverse because of variation in the distribution of fatty acids at sn-1, sn-2 and sn-3 positions. Further, absorption of fatty acids in the intestine largely depends on the fatty acid composition and their distribution in the TAGs\textsuperscript{50}. Regio-distribution of fatty acids in the BSO TAGs is shown in Table 4. The content of SDA (21.4%), LA (17.1%), OA (16%) and GLA (7%) was found to be very high at sn-2 position when compared to those at sn-1/sn-3 position (SDA, 5.4%; GLA, 1.1%; LA, 12%, OA, 12.8%). The amount of ALA (51.9%), SA (5.3%), PA (11.5%) was found to be very high at sn-1/sn-3 when compared to those at sn-2 position (ALA, 32.4%; SA, 4.2%; PA, 1.9%). Increased bioavailability of nutritionally important PUFAs such as SDA and GLA and their efficient conversion to biologically potent regula-
### Table 5  List of identified triacylglycerol molecular species in *B. arvensis* seed oil.

| S.No | Molecular formula | m/z (M+NH₄⁺) | TAG molecular species (C:DB) | Possible acyl combination of TAG identified through neutral loss of fatty acids. | Relative Intensity of total TAG (%) |
|------|------------------|--------------|-----------------------------|---------------------------------------------------------------------------------|-----------------------------------|
| 1    | C55H90O6         | 864.1        | C52:8                       | 16:0-18:4-18:4                                                                  | 1.26 ± 0.017                      |
| 2    | C55H92O6         | 866.1        | C52:7                       | 16:0-18:4-18:3                                                                  | 4.06 ± 0.027                      |
| 3    | C55H94O6         | 868.1        | C52:6                       | 16:0-18:3-18:3, 16:0-18:3-18:4                                                 | 5.03 ± 0.04                       |
| 4    | C55H96O6         | 870.1        | C52:5                       | 16:0-18:3-18:2, 16:0-18:4-18:1                                                | 4.21 ± 0.012                      |
| 5    | C55H98O6         | 872.1        | C52:4                       | 16:0-18:2-18:2, 16:0-18:3-18:1, 16:0-18:4-18:0                                 | 3.24 ± 0.013                      |
| 6    | C55H100O6        | 874.1        | C52:3                       | 16:0-18:0-18:3, 16:0-18:1-18:2                                                | 2.02 ± 0.06                       |
| 7    | C55H102O6        | 876.1        | C52:2                       | 16:0-18:2-18:0, 16:18-18:1-18:1                                               | 0.96 ± 0.001                      |
| 8    | C55H104O6        | 878.1        | C52:1                       | 16:0-18:0-18:1                                                                | 0.41 ± 0.006                      |
| 9    | C57H86O6         | 884.1        | C54:12                      | 18:4-18:4-18:4                                                                 | 0.67 ± 0.011                      |
| 10   | C57H88O6         | 886.1        | C54:11                      | 18:4-18:4-18:3                                                                  | 3.5 ± 0.019                       |
| 11   | C57H90O6         | 888.1        | C54:10                      | 18:4-18:4-18:2, 18:3-18:3-18:4                                                 | 6.35 ± 0.066                      |
| 12   | C57H92O6         | 890.1        | C54:9                       | 18:4-18:3-18:2, 18:3-18:3-18:3                                                 | 7.22 ± 0.057                      |
| 13   | C57H94O6         | 892.1        | C54:8                       | 18:4-18:3-18:1, 18:3-18:3-18:2, 18:4-18:2-18:2                                 | 7.17 ± 0.091                      |
| 14   | C57H96O6         | 894.1        | C54:7                       | 18:4-18:3-18:3, 18:3-18:3-18:4                                                 | 6.8 ± 0.116                       |
| 15   | C57H98O6         | 896.1        | C54:6                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 6.03 ± 0.053                      |
| 16   | C57H100O6        | 898.1        | C54:5                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 5.09 ± 0.0001                     |
| 17   | C57H102O6        | 900.1        | C54:4                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 3.9 ± 0.026                       |
| 18   | C57H104O6        | 902.1        | C54:3                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 2.1 ± 0.002                       |
| 19   | C57H106O6        | 904.1        | C54:2                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 0.85 ± 0.027                      |
| 20   | C59H96O6         | 918.1        | C56:9                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 1.39 ± 0.027                      |
| 21   | C59H98O6         | 920.1        | C56:8                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 3.19 ± 0.108                      |
| 22   | C59H100O6        | 922.1        | C56:7                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 4.43 ± 0.027                      |
| 23   | C59H102O6        | 924.1        | C56:6                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 3.99 ± 0.018                      |
| 24   | C59H104O6        | 926.1        | C56:5                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 3 ± 0.013                         |
| 25   | C59H106O6        | 928.1        | C56:4                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 2.02 ± 0.021                      |
| 26   | C59H108O6        | 930.1        | C56:3                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 1.27 ± 0.011                      |
| 27   | C59H110O6        | 932.1        | C56:2                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 0.75 ± 0.034                      |
| 28   | C59H112O6        | 934.1        | C56:1                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 0.4 ± 0.004                       |
| 29   | C61H104O6        | 950.1        | C58:7                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 0.82 ± 0.013                      |
| 30   | C61H106O6        | 952.1        | C58:6                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 1.59 ± 0.04                       |
| 31   | C61H108O6        | 954.1        | C58:5                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 2.17 ± 0.051                      |
| 32   | C61H110O6        | 956.1        | C58:4                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 1.7 ± 0.067                       |
| 33   | C61H112O6        | 958.1        | C58:3                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 1.2 ± 0.038                       |
| 34   | C63H114O6        | 960.1        | C58:2                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 0.61 ± 0.011                      |
| 35   | C63H116O6        | 962.1        | C60:4                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 0.28 ± 0.002                      |
| 36   | C63H118O6        | 964.1        | C60:3                       | 18:4-18:3-18:2, 18:3-18:3-18:4                                                 | 0.34 ± 0.003                      |

All the experiments were conducted in triplicate and the results are expressed in Mean ± SD.

C : DB-Carbons : Double bonds

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tors such as EPA and dihomo-GLA, respectively can be expected since the fatty acids present in the sn-2 position are more prone for absorption in the intestine. Hydrolysis of TAG in the intestine is carried out by pancreatic lipase, which is regio-specific and preferentially hydrolyze the fatty acids (FA) present at sn-1 and sn-3 position of the TAG, producing 2-MAG and FFA. In humans, the efficiency of hydrolysis of 2-MAG is very low and hence the FA in the sn-2 position remains intact as 2-MAG and gets absorbed. The re-synthesis of TAG for chylomicron assembly and transport occurs in two ways; 2-MAG pathway and phosphatidic acid pathway. 2-MAG pathway accounts for 80% of TAG synthesis in the fed state whereas phosphatidic acid pathway accounts for only 20%\(^\text{36}\)\(^\text{38}\). Hence, the fatty acid present in the 2-MAG is more readily absorbed and utilized.

3.8 FTIR spectroscopy

FTIR spectroscopy study of BSO was performed to analyse the variation in the intensity of common characteristic bands typically present in vegetable oils. The representative FTIR spectra of BSO together with chia seed oil and rice bran oil are presented in Fig. 4. Characteristic bands were assigned based on earlier FTIR studies on PUFA rich oils\(^\text{13, 23}\). A similar spectral pattern was observed in all the studied oils, yet, they showed a difference in the band intensities and the maximum absorbance frequency. This may be due to the different nature and composition of studied oils. The characteristic spectral features in the high wavenumber range of 3000-2800 cm\(^{-1}\), presenting strong triplet bands (as shown in Fig. 4) are attributed to C-H stretching vibration of methylene and methyl backbones of lipids. A distinctive band (3007-3011 cm\(^{-1}\)) in the above frequency range showed a major difference among the raw spectra of studied oils. This band is assigned to C–H stretches of cis-alkene –HC=CH– bonds and is used to determine the degree of unsaturation in oils. Further, this peak is usually found at high wavenumber for oils with more number of conjugated c=c double bonds (cis-alkene –HC=CH– bonds)\(^\text{23}\). In the current study, this band shifted from 3007 cm\(^{-1}\) for rice bran oil to 3011 cm\(^{-1}\) for BSO. This may be due to the fact that BSO has a high degree of unsaturation (i.e., fatty acids with more number of cis-alkene –HC=CH– bonds) when compared to rice bran oil. Although BSO and chia seed oil has a high degree of unsaturation, a slight shift in wave number from 3010 cm\(^{-1}\) (for chia seed oil) to 3011 cm\(^{-1}\) (BSO) was observed. This is due to the fact that BSO contains fatty acids with both 4 and 3 cis-alkene –HC=CH– bonds (i.e., SDA and ALA, respectively) (Table 1) whereas chia seed oil contains fatty acid with only 3 cis-alkene –HC=CH– bonds (ALA). The new unsaturated fatty acid (SDA) found in BSO may be
responsible for a slight shift in wave number from 3010 cm\(^{-1}\) to 3011 cm\(^{-1}\).

In the lower wavenumber region, a peak at 1743 cm\(^{-1}\) was observed in all studied oils. This peak is assigned to carbonyl (O = C) stretching of ester functional groups found in fatty acids and lipids and hence, provides a measure of total lipids in the oil. A weaker band near 1653 cm\(^{-1}\) is attributed to C = C stretching vibration of cis-olefins and its intensity can also be used to determine the degree of unsaturation\(^{30}\). Similar to the pattern observed at the wavenumber 3011 cm\(^{-1}\), the peak intensity at 1653 cm\(^{-1}\) also indicates that BSO has a high degree of unsaturation when compared to chia seed oil and rice bran oil. Overlapped bands in the wavenumber range of 1470-1370 cm\(^{-1}\) arose due to the coalition of deformation modes of methylene and methyl groups. The strong peak at \(\sim 1160\) cm\(^{-1}\) is assigned to a combination of asymmetric stretching modes of (C–C (=O)–O) and (O–C=O) bonds present in ester and ether structures. In addition to this, another strong peak at 999 cm\(^{-1}\) was observed in the studied oils and is assigned to symmetric stretches of C-O-C bonds found primarily in triglycerides, cholesterol esters and ethers. At the lower region of the spectra, an intense band at 720 cm\(^{-1}\) was assigned to the coalition of rocking vibration and out-of-plane deformation of \(-\text{CH}_2\) group in cis-di-substituted olefins\(^{30}\). Overall, FTIR spectra of BSO revealed the entire common characteristic molecular features found in vegetable oils and can be used to determine authenticity of the oil.

4 Conclusion

The current study has shown the characterization of Indian grown \(B. \text{ arvensis}\) seed oil through the analysis of physico-chemical parameters, total lipids and their fatty acid distribution, profiling of tocopherols and TAG molecular species for the first time. The seed oil has been classified as ALA-GLA-SDA oil or \(\omega\)-6-\(\omega\)-3 oil with more than 81.3% PUFA. Our results of lipid class profiling indicate neutral lipid as predominant lipid class with 78.8% PUFA. \(\gamma\)-tocopherol, an efficient antioxidant was found to be the predominant tocopherol isomer in BSO. Owing to high PUFA content, BSO gets oxidized rapidly requiring an efficient method to prevent oxidative damage during its processing, transportation and storage. More than 70% of TAG molecular species contain PUFA, which are mostly found on TAG backbones with more monounsaturated fatty acids than with saturated fatty acids. SDA and GLA are majorly found on the sn-2 position of TAG and hence more prone for absorption in the intestine. FTIR spectroscopy analysis of BSO showed a high degree of unsaturation than CSO and RBO, which was evident by the presence of the fatty acid with four double bonds (SDA). Overall, the results of the present study not only suggest the potential of BSO as health promoting dietary oil but also its application as an omega-3 supplement for food and nutraceutical industries.

Authors Contribution

R. V. Sreedhar and P. Prasad conceived and designed the study. L. Prasanna Anjaneya Reddy and P Prasad cultivated the plants. P. Prasad and S. Savyasachi performed the experiments. P. Prasad and R. V. Sreedhar wrote the manuscript. All the authors reviewed and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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