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ORIGINAL PAPER

Profiling of Lipids, Nutraceuticals, and Bioactive Compounds Extracted from an Oilseed Rich in PUFA

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Abstract

Polyunsaturated fatty acid (PUFA) rich vegetable oils are nutritionally and economically important agriculture based commodities. The lipid profile, nutraceutical content, and antioxidant activity of Indian chia seed oil (CSO) were analysed and compared with PUFA rich vegetable oils. The total oil content was 28% (w/w) with α-linolenic acid (ALA; 65%) as the predominant fatty acid and a n-3/n-6 ratio of 3.5. The tocopherol content was 144 mg/kg of oil, with $\gamma+\beta$ being the most abundant. The squalene content was 178.47 mg/100 g of oil, and the total phenolic content was 0.014 mg GAE/g of oil. The identity of major polyphenols in the methanolic extract of CSO were established by LC-HRMS. FTIR spectra of CSO exhibited characteristic features that were identical to other PUFA rich oils. Results demonstrate that the Indian CSO is an excellent source of essential fatty acids and key nutraceuticals.

Keywords Chia seed oil · Lipid profiling · Nutraceuticals · PUFA

Introduction

Oils constitute a major part of the human diet. The lipid profile of the individuals is largely governed by the type and quantity of fats present in their food sources [1]. Consumption of foods rich in cholesterol, saturated and trans fat leads to an increased risk of chronic diseases, including coronary heart disease, diabetes, and cancer [2]. The consumption of foods rich in unsaturated fatty acids, such as omega-3 PUFAs is considered as a versatile mediator for improving and maintaining human health over the entire lifespan [3]. Further, omega-3 fatty acids have also been shown to play an important role in brain development, cognition, behaviour, and to reduce coronary heart disease risk [4]. Few PUFAs are essential fatty acids (linoleic acid (LA) and α-linolenic acid (ALA)) and must be obtained in the diet or dietary supplements as humans cannot synthesize them *de novo* to the required levels. Primary sources of ω-3 PUFAs are marine (fish) and plant based oils. The worldwide consumption of fish oil was 2,82,000 t in 2014, and is expected to reach 7,11,000 t by 2025. However, the consumption of fish based sources is limited due to the affordability issues [5]. Dietary supplements of PUFA, available in the market, are costly. To meet the daily global requirements, there is a need to generate feasible plant based alternatives. Seed oils derived from the plant kingdom (Flax, chia, buglossoides, Hemp, and Walnuts) are an economical, acceptable, and sustainable source of omega-3 PUFAs [6, 7].

Salvia hispanica (chia) is the highest reported terrestrial plant source of ω-3 fatty acid, ALA. It is a herbaceous plant belonging to the mint family Lamiaceae and is grown commercially in Bolivia, Argentina, Guatemala, Mexico, and India. Consumption of chia seeds has been increasing over the years, owing to its health benefits in reducing obesity, diabetes, cardiovascular diseases, certain types of cancer, and due to possessing significant antioxidant activity [8–10]. The seeds are a good source of essential fatty acids, proteins (17–24%), dietary fibers (18–30%), minerals, and antioxidants [11]. Chia seeds contain a total PUFA content of >80%. Since the n-3/n-6 ratio of the seed oil is 3.5, its regular consumption can assist in maintaining a healthy dietary ratio. Chia seeds are nutritionally dense and bioactive rich and are being increasingly included in the functional foods (yogurt, bread) to benefit human health [12–14].

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In general, the oil content and fatty acid composition of oilseed crops like chia vary due to various factors such as geographical locations, agricultural practice, climatic condition, and cultivar or the variety of the plant [11]. The oil content of chia in different regions of the world varies from 25 to 40% [15], and the trilinolenin content of seeds varied from 33 to 54% [16, 17]. The only study on seed oil of chia grown in India assessed its anti-inflammatory property and physicochemical characteristics [18]. The present study was carried out to investigate the oil content, the fatty acid composition of different lipid classes, positional distribution of fatty acids, tocopherols, phenolics, sterol content, and antioxidant activities of a high yielding chia cultivar (CHIAmpion B-1) grown in Southern India, and compared with other vegetable oils.

Materials and Methods

The materials and methods are presented as supplementary material.

Results and Discussion

Oil Content and Fatty Acid Composition of Indian Chia Seeds

The fatty acid composition of CHIAmpion B-1 compared with chia grown in Australia and South American countries are shown in Table 1 [15–17]. The fatty acid profile obtained was very similar to those grown in other countries, indicating that cultivation in tropical climatic conditions of India does not have much effect. ALA was the predominant fatty acid in all regions (>60%), followed by LA (18–20%), and the overall PUFA content was >80%. Oleic acid (MUFA) constituted 6%, while saturated fatty acids (SFA), palmitic and stearic acids, constituted 6 and 2%, respectively.

The PUFA/SFA ratio of Indian CSO was 9.3, similar to the ratio observed in other countries. UFA/SFA ratio and the ω-6/ω-3 ratio of Indian CSO were 9.9 and 0.29, respectively (Table 1). As *per* the Wijendran and Hayes report, the 6:1 ratio of the ω-6/ω-3 fatty acids intake accelerates the risk reduction of chronic diseases, including inflammation and autoimmune diseases [19]. Therefore, the consumption of Indian chia seeds will help maintain a healthy dietary ratio of fatty acids. Oilseed crops that produce an oil content of >15% w/w (of dry weight) are generally considered economically viable for commercial oil production [20]. The CHIAmpion B-1 cultivar of chia, grown in tropical climates of South India, produced an oil content of 28% (dry weight basis). The obtained oil yield is similar to those reported for chia grown in Australia, Mexico, Guatemala, and Bolivia. The most abundant TAG species found in Indian CSO was trilinolenin–αLnαLnαLn, 44%, which is unique as it has ALA at all three positions of TAG [21]. The second most abundant TAG was αLnαLαLn, 21%. Similarly, the Australian and Mexican seeds produced 33 and 54% trilinolenin, respectively [15–17].

Fatty Acid Composition of Lipid Classes in Indian CSO

Lipid classes of CSO were separated *by silica* column chromatography [22]. Lipid class determination is important as they affect the quality of oils due to their distinct fatty acid composition. Few studies show that the identification and characterization of polar lipid class provide valuable information in detecting oil adulteration [23]. Among the three lipid classes in CSO, neutral lipids (91.4%) were found to be most abundant, followed by phospholipids (4.8%), and glycolipids (3.8%). PUFAs (LA & ALA) were the most

Table 1 Fatty acid profile of CHIAmpion B-1 (Indian chia variety), in comparison with seeds grown in other parts of world

Oil yield / Fatty acids (μmol %)	CHIAmpion B-1 (India)	Mexico[#]	Australia^	Argentina*	Guatemala*
Oil content (%)	28	31.8	26.6	33.6	26.7
Palmitic acid (C16:0)	6.55 ± 0.09	7.07 ± 0.05	6.21 ± 0.05	6.2 ± 0.4	5.5 ± 0.1
Stearic acid (C18:0)	2.64 ± 0.05	2.81 ± 0.04	1.89 ± 0.01	3.0 ± 0.7	2.7 ± 0.2
Oleic acid (C18:1)	6.23 ± 1.1	5.50 ± 0.01	5.68 ± 0.05	5.3 ± 1.1	5.8 ± 0.3
Linoleic acid (C18:2, n-6)	18.66 ± 1.5	19.8 ± 0.09	21.46 ± 1.24	19.7 ± 0.0	16.6 ± 1.2
α-Linolenic acid (C18:3, n-3)	65.91 ± 1.6	63.64 ± 0.08	64.3 ± 2.31	65.6 ± 0.8	69.3 ± 1.0
Total SFA	9.1	10.16	8.5	9.3	8.3
Total MUFA	6.23	5.5	5.68	5.3	5.8
Total PUFA	84.56	83.48	85.8	85.4	85.9
n-3/*n*-6 ratio	3.5	3.21	3.0	3.32	4.1

Experiments were conducted in triplicates, and results were expressed as mean ± SD

([#]Shen et al. [17], ^Timilsena et al. [16], *Ixtaina et al. [15])

abundant fatty acids in all lipid classes and constituted ~70% of total fatty acids present. Neutral lipids had the highest PUFA content (82.4%), followed by glycolipids (78.6%) and phospholipids (69.2%). All lipid classes contained ~8% of MUFA (OA), while SFA were most abundant in phospholipids (22%), followed by glycolipids (14%), and neutral lipids (10%) (Supplementary Table 1).

Positional Distribution of Fatty Acids in Indian CSO

The TAG molecular profile of each oil is highly unique due to the different combinations of FAs possible in each TAG species. Furthermore, the characteristic effects of TAGs on digestion, absorption, and transport are closely associated with the fatty acid binding positions [24]. TAG is hydrolyzed by the enzymatic activity of pancreatic lipase, which is regiospecific and cleaves preferentially at *sn*-1 and *sn*-3 positions to form 2-Monoacylglycerol and free fatty acids (FFA) [15]. Regio distribution of CSO TAGs is given in Table 2.

Both LA and ALA were found predominantly at *sn*-2 position in Indian CSO (Table 2). However, SFA were more predominant at the *sn*-1/*sn*-3 position (PA-18% and SA-10%). The profile obtained correlated with Mexican CSO, which possessed 63% ALA and 25% LA at its *sn*-2 position [17]. A high proportion of PUFA at the *sn-2* position in CSO helps increase bioavailability, as they are easily absorbed in the human intestine.

Phytosterol Content

Phytosterols are one of the most important nutraceuticals present in vegetable oils. All vegetable oils usually contain phytosterols and squalene. Phytosterols play a major role in reducing the concentration of low density lipoproteins (LDL) in blood and also help to minimize the risk of cardiovascular diseases [25]. Squalene can act as an antioxidant, anti-cancer compound, and can also be used as a skin lubricant. It is also known to reduce cholesterol levels. In Indian CSO, 136.3 mg/100 g of ergosterol and 219.2 mg/100 g of stigmasterols were present (Table 3). The vegetable oils usually possess minor amounts of squalene, but Indian CSO contained 178.47 mg/100 g of oil. This high squalene content may contribute to the beneficial effects of CSO on human health. In contrast, Mexican CSO contained only 226.6 mg/kg of squalene [17]. The squalene content of Indian CSO is substantially higher than the concentration present in edible peanut, walnut, and almond oils [26].

Tocopherol Content

Tocopherols act as natural antioxidants and possess free radical scavenging activity [26]. They are lipid soluble and found in minor quantities in all seed oils. The tocopherol content in Indian CSO was ~144 mg/kg of oil. Oils having high PUFA content are usually associated with high amounts of tocopherols. $\gamma+\beta$-tocopherols were the most abundant (126.7 mg/kg of oil), followed by the δ-tocopherol (12.9 mg/kg), while the α-tocopherol was the least abundant (4.8 mg/kg of oil) in Indian CSO (Table 3). The tocopherol profile obtained was shown in Fig. 1. γ-tocopherols exhibit the strongest antioxidant potential, and was also the predominant class in other vegetable oils such as hemp oil, rapeseed oil, and sacha inchi [26, 27].

The tocopherol content of Indian CSO was comparatively higher than the Mexican variety (76.9 mg/kg), but it was less than the CSO from Argentina, which contained 238 –427 mg/kg of tocopherols [15]. The difference in content could be due to geographical conditions.

Phenolic and Polyphenols Content

Phenolics have many beneficial and medicinal properties against chronic diseases, including cancer, neurodegeneration, and cardiovascular diseases [28]. The total phenolic content was analyzed by the Folin-Ciocalteu method. The total phenolic content was determined using a methanolic extract of CSO, acidified with 0.1% HCl. Indian CSO extract showed a phenolic content of 0.014 mg GAE/g, lower

Table 2 Fatty acid composition and positional distribution of fatty acids in triacylglycerol

Fatty acids (μmol%)	Monoacylglycerol (*sn*-2)	Free fatty acids (*sn*-1/*sn*-3)
Palmitic acid	3.75 ± 0.4	18.2 ± 0.5
Stearic acid	1.1 ± 0.2	10.5 ± 0.3
Oleic acid	14.2 ± 2.4	7.6 ± 0.4
Linoleic acid	28.6 ± 0.4	15 ± 0.2
α-linolenic acid	52.4 ± 2.5	48 ± 1.4

Experiments were conducted in triplicates, and results were expressed as mean ± SD

Table 3 Nutraceutical composition of Indian CSO

Phytosterols (mg/100 g of oil)	
Ergosterol	136.31 ± 1.7
Stigmasterol	219.2 ± 2.4
Squalene	178.47 ± 2.3
Tocopherols (mg/kg of oil)	
α- tocopherol	4.8 ± 0.6
$\gamma+\beta$- tocopherol	126.7 ± 2.9
δ- tocopherol	12.9 ± 1.7

Experiments were conducted in triplicates, and results were expressed as mean ± SD

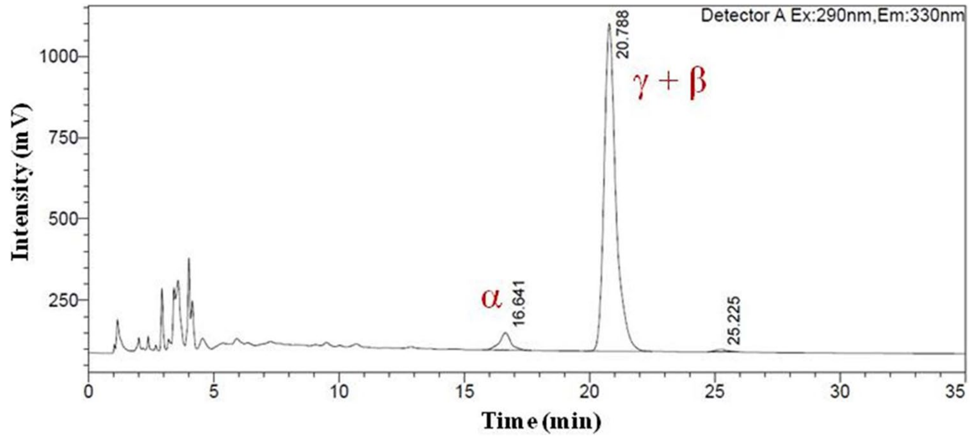

Fig. 1 HPLC chromatogram of identified tocopherols in Indian CSO

than the value exhibited by Brazilian chia seeds, 0.97 mg GAE/g [29]. The differences in total phenolic content can be attributed to climate, cultivation techniques, and methods employed to determine phenolic content.

The diverse polyphenols present in the CSO were analysed by qTOF-MS (Fig. 2). The methanolic extract of CSO consisted of citric acid, caffeic acid, ferulic acid, hydroxygallic acid, coumaric acid, and ethyl protocatechuate.

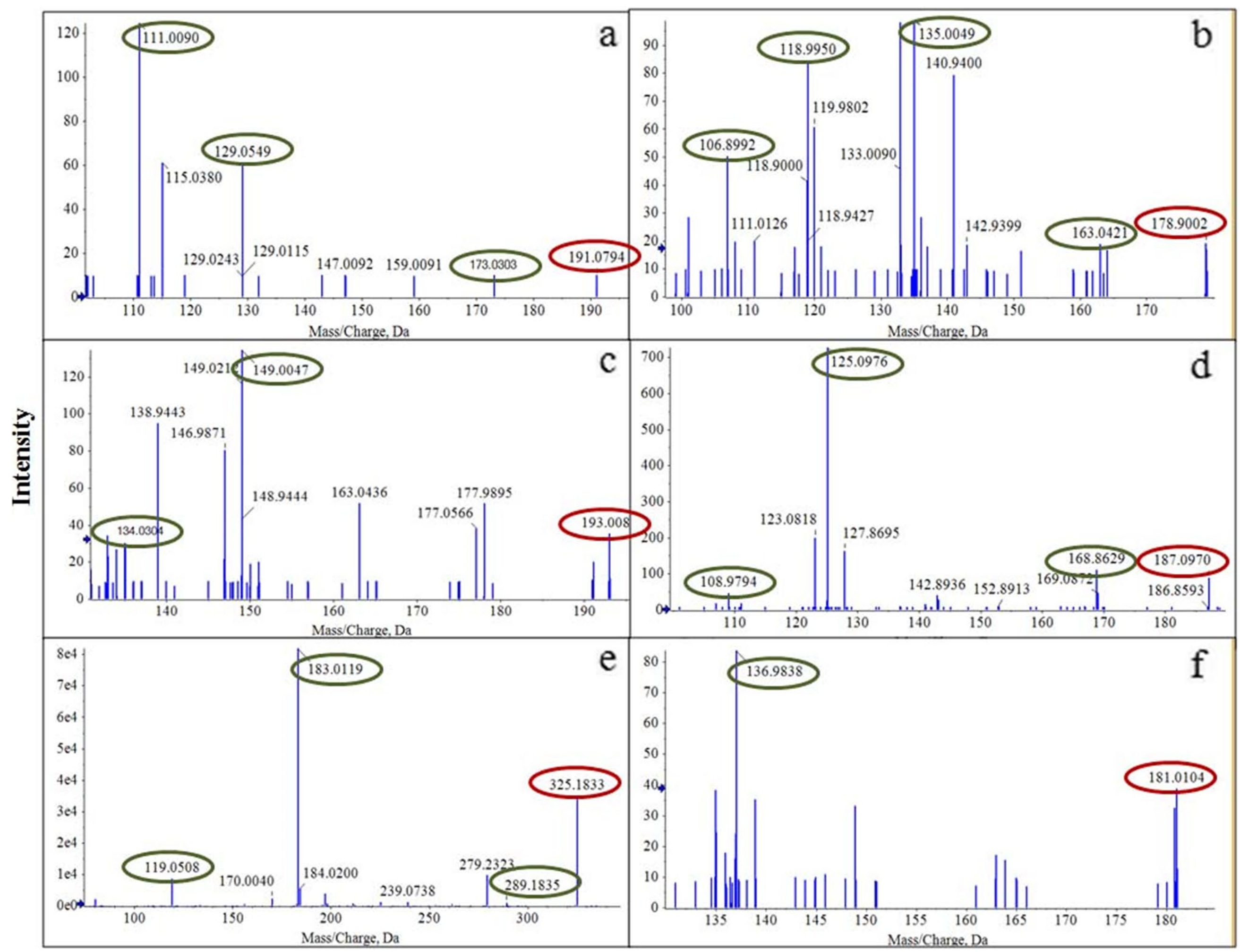

Fig. 2 MS/MS fragmentation pattern of identified polyphenols in Indian CSO. **a**) Citric acid **b**) Caffeic acid **c**) Ferulic acid **d**) Hydroxygallic acid **e**) Coumaric acid **f**) Ethyl protocatechuate. The precursor (red) and product (green) ions are colored

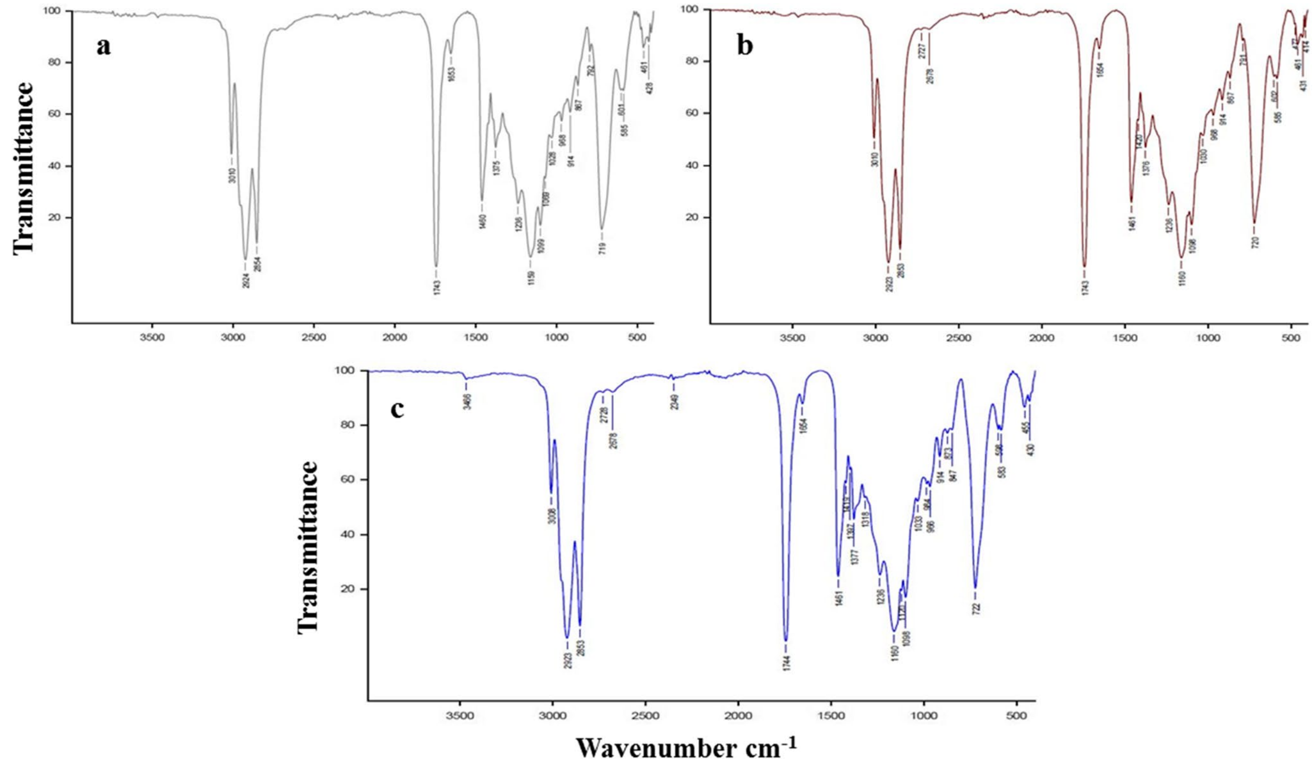

Fig. 3 FTIR spectra of **a**) Indian CSO, in comparison with **b**) flaxseed oil and **c**) rice bran oil

These compounds were directly identified by comparing the obtained mass with authentic standards. Post identification, the structures of polyphenols were confirmed by comparing the accurate mass and typical MS fragmentation with standards. Supplementary Fig. 1 contains the total ion chromatogram (TIC) profile of CSO, and Supplementary Table 2 explains the identified polyphenols and their MS fragmentation pattern.

Thermal Behaviour

Differential scanning calorimetry (DSC) was used to analyze the thermo-oxidative process of CSO as a function of temperature and time. Crystallization peak was not very prominent when CSO was cooled down to −50 °C, but upon heating the oil, a single prominent phase transition starting from −32.78 °C and ending at −28.8 °C with a peak at −30.7 °C was observed in the melting curve (Supplementary Fig. 2). Melting of TAG molecules depend on fatty acid chain length, the extent of chain branching, degree of unsaturation of its constituent fatty acids, and their stereo-specific distribution. Oils rich in unsaturated fatty acids undergo melting at a lower temperature, which was observed in Indian CSO. The curve observed is also relatively flat above 0 °C, indicating that CSO is a pure liquid at ambient temperature without any detectable solid crystals. Similarly, Australian CSO also showed a single peak at −34 °C, and the curve obtained was also flat above 0 °C [15].

FTIR Spectroscopy

The Indian CSO was subjected to FTIR to study the variation in the intensity of characteristic bands seen in vegetable oils. The CSO FTIR spectra, along with flax and rice bran oil, are shown in Fig. 3. The bands are assigned by studying the FTIR spectra of other PUFA rich oils (Supplementary Table 3). The highest peak in chromatograms was at 2934 and 1743 cm^{-1}, which indicates the linear relationship between the numbers of *cis*-double bonds for a series of unsaturated fatty acid methyl esters and –C=O carbonyl stretching mode of ester functional groups in lipids. The distinct peak obtained at 3010 cm^{-1} indicates the C-H stretching of cis-olefinic –HC=CH- bonds. This peak at a high wave number indicates a high concentration of unsaturated fatty acids (ALA) and the presence of conjugated (C=C) double bonds in Indian CSO. In the present study, this band shifted from 3008 cm (rice bran oil) to 3010 cm^{-1} (Indian CSO), highlighting the fact that CSO had a higher degree of unsaturation (more number of *cis*-alkene –HC=CH– bonds), in comparison with rice bran oil. Similarly, Australian and Mexican CSO also produced this distinct band at 3010 cm^{-1}

[15, 16]. Oils of marine origin examined gave this peak at higher wave numbers (3013 –3015 cm^{-1}) due to the presence of Ecosapentaenoic acid (EPA, C20:5) and Docosahexaenoic acid (DHA, C22:6), which contain 5 and 6 conjugated double bonds, respectively [30]. A peak at 1743 cm^{-1} (lower wavenumber region) was observed in all oils, and this assigns to the carbonyl stretching of ester functional groups present in fatty acids. This provides a measure of total lipids present in oils. The distinct weak band at 1654 cm^{-1} refers to the C=C stretching vibration of *cis*-olefins, and its intensity measures the high degree of unsaturation present in CSO. The absorption band at 1154 cm^{-1} represents the saturated ester band (C-C(=O)-O), which is characteristic of vegetable oils. The absorbance at 2823 cm^{-1} indicated the stretching of the –C-H (CH_2) groups. The CH_2 bending of the acyl chains of lipids and CH_2 scissoring vibration of the acyl chains was observed at 1461 cm^{-1}. The absorbance between 750 to 717 cm^{-1} indicated the presence of overlapping methylene (-CH_2) rocking vibration and the out of plane vibration of *cis*-disubstituted olefins. The FTIR spectra showed that the Indian CSO functional groups correlated with chia seed oil reported in other regions.

Conclusions

The Indian CSO is an excellent source of ω-3 fatty acids, with LA and ALA constituting >80% of total fatty acids. The seeds produced 28% w/w oil content, and the ω-3 to ω-6 ratio was 3.5:1. PUFAs were more predominant at the *sn*-2 position and could probably be more efficiently absorbed in the intestine. FTIR analysis also confirmed a high degree of unsaturation in CSO. Squalene content (178.4 mg/100 g of oil) was very high, and this can help prevent cancer, act as an antioxidant, used as skin lubricant, and reduce cholesterol levels. Overall, the usage of Indian chia seeds should be stimulated as it has high nutritional value and contains bioactive compounds that impart health benefits to humans compared to other vegetable oils.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11130-021-00945-0.

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Data Availability All data generated or analysed during this study are included in this published article and its supplementary information files.

Declarations

Ethics Approval This article does not contain any studies with human or animal objects.

Conflict of Interest The authors declare that they have no conflict of interest.

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