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RESEARCH PAPER

Shotgun approach reveals distinctive lipid profiles in brassica oilseeds: A high-resolution ESI-ToF-MS study

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Abstract

Members of Brassica seed oil are important sources of nutritionally superior edible oil. There are no comprehensive reports on complete lipidomic profile of these oilseed crops. In this study, the lipidomic profiling of edible oil from 7 different cultivated species of Brassica seed oils was performed by shotgun approach using electrospray High Resolution Time of Flight-Mass Spectrometry (ESI-ToF-MS). The mass spectrum under positive polarity revealed 1098 lipids under different lipid classes including sphingolipids, phospholipids and different storage lipids. Under negative polarity, 70 lipids including free fatty acids (FFA), cardiolipins and phospholipids were detected. Erucic acid in FFA form was found to be most abundant in both Yellow and Brown sarson. *Brassica napus* contains almost all forms of cardiolipins (CL). Out of 26 different species of cardiolipins detected in negative ion mode, CL 56:1 (FA 18:1) and CL 56:1 (FA 22:1) were present only in brown sarson. Similarly, CL 56:2 (FA 18:2) and CL 56:1 (FA 22:1) were present only in Yellow and Brown sarson. These findings enhance our understanding of the nutritional diversity in Brassica seed oils, emphasizing the significance of lipidomic analysis for elucidating the molecular composition of edible oils.

Keywords

Brassica, Edible oil, Global lipidomics, Shotgun Lipidomics, ToF-mass spectrometry

Introduction

Rapeseed-mustard oil is the third most consumed edible oil in the world after soybean and palm oil (Shen et al. 2023). Brassica seed oil is a collective term used for related oil yielding species belonging to the family Brassicaceae. The major crops are *Brassica napus* L. (rapeseed), *Brassica juncea* L. (Indian mustard), *Brassica rapa* L. var. Yellow sarson, *Brassica rapa* L. var. Toria, *Brassica rapa* L. var. Brown sarson, *Brassica carinata* A. Br. (Ethiopian mustard) and *Eruca sativa* Mill. (taramira).

Rapeseed-mustard oil is one of the most preferred and healthy edible oil as they are low in saturated fatty acids. They are preferred also because they are high in essential fatty acids which are nothing but polyunsaturated fatty acids (PUFAs) belonging to ω3 and ω6 families. The significance of ω3 fatty acids in human health is well-established through epidemiological studies linking an ω3-rich diet to the prevention of diseases like cardiovascular diseases and myocardial infarction (VonSchacky and Harris 2007). Research indicates three primary effects of ω3 fatty acids on cardiovascular health: anti-arrhythmic (Leray et

al. 2001), hypolipidemic (Buchner et al. 2002), and antithrombotic (Albert et al. 2002), leading to reduced arteriosclerosis. There are reports that indicate that omega-3 oils offer health advantages in various other domains, including premature infant health (Carlson 1999), asthma (Broughton et al. 1997), bipolar and depressive disorders (Calabrese et al. 1999), as well as conditions like dysmenorrhea and diabetes (Connor 2000). Alpha-linolenic acid (ALA) is one of the major omega-3 fatty acids that is present abundantly in flaxseed and found in small amounts in hemp, walnut, soybean, and canola oil (Hunter 1990).

Linoleic acid serves as the primary ω6 polyunsaturated fatty acid (PUFA), while arachidonic acid, a longer-chain ω6 PUFA, constitutes approximately 2% of total PUFA. Arachidonic acid is derived from the metabolism of linoleic acid and acts as the precursor for various reactive oxygenated metabolites. Linoleic acid plays a crucial role in maintaining the structural integrity of the skin and supporting barrier function, as it is an essential component of ceramides (Rabionet et al. 2014). Moderate intake of linoleic acid, when used as a partial replacement for saturated fatty acids, has been shown to lower both total cholesterol and low-density lipoprotein (LDL)-cholesterol concentrations in the blood (Froyen et al. 2020). Arachidonic acid can constitute up to 25% of the fatty acids in phospholipids found in skeletal muscles, brain, liver, platelets, and immune cells (Calder 2007). Beyond their role in regulating immunity and inflammation (Calder 2020), eicosanoids derived from arachidonic acid are also involved in the regulation of platelet aggregation, hemostasis, thrombosis, and vascular tone (Crescente et al. 2019).

Brassica seed oils are also rich in phytosterols (1–1.5 %), tocopherols and β-carotene (Ghazani and Marangoni 2013). Nutritional profiling of these crops has been mostly concentrated on either fatty acid profile or other targeted compounds. There are no comprehensive reports on the complete profiling of these oils. Hence a complete lipidomic profiling of these oils will be helpful in identifying the best sources for nutritionally beneficial mustard oil. Although these species are related to each other, considerable variation can be observed in their biochemical repertoire and nutritional profile. For example, the canola type rapeseed which is *Brassica napus* genotype with low erucic acid content (less than 2 %) are more preferred as erucic acid has been considered antinutritional in nature as it is said to cause myocardial lipidosis mostly in rodents fed with high erucic acid diet (Kramer et al. 1992; Pasini et al., 1992; Badawy et al. 1994). However this effect is transient and reversible even after prolonged intake. Especially in humans erucic acid induced lipidosis has not been described (Knutsen et al. 2016). There are reports that dietary erucic acid therapy was effective in lowering plasma C26:0 to normal in adrenoleukodystrophy (ALD) patients, and that this therapy might prevent further demyelination in some mildly affected patients (Asano et al. 1994; Cappa et al. 2012). Huge variation (0–52 %) in erucic acid content can be observed in rapeseed-mustard oils. Same is the case with saturated fatty acids (5–7 %) and polyunsaturated fatty acids with 7–10 % α-linolenic acid and 17–21 % linoleic acid (Pellet et al. 2008; El-Beltagi and Mohamed 2010). Nuclear Magnetic Resonance (NMR) metabolomic studies of *B. rapa* and *B. napus* reveal that PUFA content is more in *B. rapa* (Kortesniemi et al. 2015). The present study was intended to see the variation in the lipid composition of different cultivated species of rapeseed-mustard.

A number of methods have been utilized in rapeseed-mustard species for metabolomic and lipidomic studies. Non-targeted metabolomic analysis via Ultra Performance Liquid Chromatography-Quadrupole Time of Flight Mass Spectrometry (UPLC–QTOF–MS) has been utilized in *B. napus* (Farag et al. 2013). 1H NMR metabolomics was used in *B. rapa* and *B. napus* to see the differences in major lipids and minor metabolites between the two species (Kortesniemi et al. 2015). Variation in fatty acid composition among different genotypes of *B. napus* has been studied using Gas chromatography-mass spectrometry (GC-MS) (El-Beltagi and Mohamed 2010). Low and high erucic lines were successfully identified using 1H NMR metabolomics in *B. napus* (Han et al. 2016). Zheng et al. 2017 have done the glycerolipid profiling of yellow sarson by using UPLC coupled to triple time-of-flight mass spectrometry (UPLC-Triple-TOF-MS).

Analysis of lipids using mass spectrometry plays crucial role in the field of lipidomics (McDonald et al. 2016). Direct infusion-based shotgun approach is suitable for high throughput analysis of lipids as it provides direct MS scans and or specific precursor ion scans (PIS), neutral loss scans (NLS), selected ion monitoring (SIM) or data dependent MS scans for detecting lipid species (Xie et al. 2019). The most commonly used method for MS nowadays uses the atmospheric pressure chemical ionization (APCI) source either without separation or coupled with liquid chromatography especially for less polar lipid classes (Holcapek et al. 2018).

Metabolite profiling with reference to flavors have been done using gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactory (GC-O), and sensory analysis in unheated delicate fragrance rapeseed oil (DFRO), refined rapeseed oil (RRO), unheated strong fragrance rapeseed oil (SFRO) and umami fragrance rapeseed oil (UFRO) by Guo et al. (2023).

Raza et al. (2021) have discerned 31 differentially accumulated metabolites (DAMs) in rapeseed (*Brassica napus*), using metabolome analysis, when comparing cold-tolerant and cold-sensitive varieties. Liquid Chromatography- Mass Spectrometry (LC-MS/MS) analyses were carried out by them utilizing a UHPLC system with a UPLC BEH Amide column coupled with a TripleTOF 6600 (Q-TOF, AB Sciex).

However, when considering only lipids, currently there are two complementary approaches in lipidomics by mass spectrometric analysis such as shotgun lipidomics (direct infusion) and the use of liquid chromatographic separations before analysis. Shotgun lipidomics facilitate the high-throughput global analysis of lipidome by exploiting the chemical and physical properties of lipids directly from the sample. In shotgun lipidomics, the mass spectrum discloses molecular ions of individual lipid species of in-

terest allowing precursor-ion scans of the particular fragment ions for their identification and quantitation. Each scan cycle establishes the identity of the molecular ion by recognizing the building blocks of each lipid class (Wang et al. 2016). In the present study, the shotgun approach of lipidomics has been utilized to identify and determine the relative abundance of major lipid species in mustard oils obtained from different related species.

Materials and methods

The study was conducted at the ICAR-Directorate of Rapeseed Mustard Research (DRMR) in Bharatpur, India, situated at coordinates 27°11'N, 77°27'E. The climate in this area is predominantly semi-arid, characterized by long hot summers, brief intermittent rainfall during the south-west monsoon, and a winter period lasting almost five months, with average temperatures dropping below 22.5 °C. The winter season often aligns with one or two instances of rain attributed to western disturbances. The soils in the region are primarily deep, well-drained inceptisols with a texture ranging from coarse to fine loamy.

The crops were grown during 2015–16 season. N, P, and K at rates of 80:40:40 kg/ha, as well as sulphur at rates of 40 kg/ha, zinc sulphate at rates of 25 kg/ha, and borax at rates of 10 kg/ha was applied. Half of the nitrogen was applied as a basal dosage and the remaining half during the first irrigation at 30 to 45 days after sowing. Irrigation was given at pre-flowering and siliquae forming stages. After harvesting the seeds were stored at room temperature at the germplasm collection of Indian Council of Agricultural Research (ICAR) - Directorate of Rapeseed-Mustard Research, Bharatpur situated in the semiarid regions of Rajasthan state of India. Oil extraction was done in January 2017 and it was stored at 4 °C in 3 ml glass vials with lids till the MS analysis was done within a week.

Mustard species and chemicals

Seven different cultivated species of Brassica seed oil including *Brassica juncea* (RH-749), *Brassica rapa* var. Yellow sarson (NRC-YS-05-2), *Brassica rapa* var. Toria (PT-30), *Brassica rapa* var. Brown sarson (DRMR-388), *Brassica napus* (GSL-1), *Brassica carinata* (PC-5) and *Eruca sativa* (DRMR-171) were chosen for the study.

n-Hexane (95 %), methanol, dichloromethane (DCM), ammonium acetate buffer (purchased from SRL pvt ltd, India).

Extraction of oil

1 g of seeds was crushed using pestle and mortar and placed in extraction thimbles. Extraction was carried out in a Soxhlet apparatus (macro scientific) for 4 hours at 70 °C using n-hexane (Sisco Research Lab) (95 %). The solvent was evaporated using a rotary evaporator (Heidolph). The oil thus obtained was stored at 4 °C till it was taken out for analysis. The oil recovery ranged from 35 to 42 %.

Sample preparation prior to analysis

The crude oil obtained by Soxhlet extraction process was diluted 10-fold with methanol: dichloromethane (DCM) (Sisco Research Lab) (50:50 V/V) followed by second dilution of 10-fold in 10 mM ammonium acetate (Sigma Aldrich) in methanol. Finally, 5 µl diluted oil was dissolved in 5 ml of dichloromethane: methanol (40:60) solvent mixture along with ammonium acetate buffer and analyzed on a High-Resolution Quadrupole Time of Flight-Mass Spectrometry (QToF-MS).

LC method and mass spectrometry acquisition parameters

50 µl of each sample were analyzed in replicate injection (n = 6) using Flow Injection Analysis (FIA) by Shimadzu Prominence autosampler (Shimadzu Corporation Kyoto, Japan) and isocratic pump. Isocratic mobile phase as 98 % methanol with 2 % water (5 mM ammonium acetate) was used to push sample to the source at a flow rate of 10 µl/min. Nebulizing gases GS1 and GS2 were kept at 20 psi and 15 psi, respectively. Curtain gas was set at 15 psi, positive mode ion spray voltages at 5500 V, negative mode ion spray voltages at 4500 V, declustering potential at 40V and ESI source temperature was operated at 300 °C. The method has been reproduced later by Raza et al. (2021).

MS/MS^{ALL} with flow injection analysis (FIA) coupled with triple TOF mass spectrometry

Triple TOF system was calibrated for MS and MS/MS in both positive and negative mode using the standard calibration reagent before acquiring each sample for mass accuracy and resolution. Diluted mustard oil lipid extracts were introduced by direct infusion using Shimadzu 20AD chromatography system (Shimadzu Corporation Kyoto, Japan). A previously reported method (Denke 2006) was followed for instrument setting. The Q1 quadrupole was set to step 1 Da increments across the mass range for selecting lipid precursors at unit resolution. For complete Collision Induced Dissociation (CID) fragmentation, the isolated precursor ions were passed through the collision cell. A high resolution ToF-MS scan was also included in the cycle for high mass accuracy. In the current study MS/MS^{ALL} with FIA workflow on the Triple-TOF 6600 (AB Sciex, Concord Canada) system was activated by MS/MS^{ALL} mode tool in the AnalystTF ® 1.7 software.

The masses isolated in Q1 were derived from those that had appropriate mass defects set for both positive and negative ion modes. Finally, a TOF-MS scan ranging from

200–1500 m/z with accumulated time for 250 ms followed by acquisition of ~1000 product ion spectra from Q1 mass 200 to 1200 m/z stepping by 1 Da was included. MS/MS spectra were acquired from 100–2000 m/z accumulated for 100 ms each and the total cycle time for the experiment was set at 1.8 min each for positive and negative polarity.

Lipid species identification using LipidView™ software

Data processing was done using LipidView™ 1.32 Software (AB Sciex, Concord Canada) which process high resolution TOF-MS and MS/MS data in the Infusion MS/MS^{ALL} workflow in both polarities. De-isotoping correction was applied for high resolution which provides more accurate response for the identified lipid species. Peak intensities or peak area measurements was then corrected or normalized. Lipid view 1.2 software was used for viewing the precursor masses of all fragment ions that make up the lipid species. Results of positive and negative modes of infusion MS/MS^{ALL} workflow were compared among oils from seven different rapeseed-mustard species.

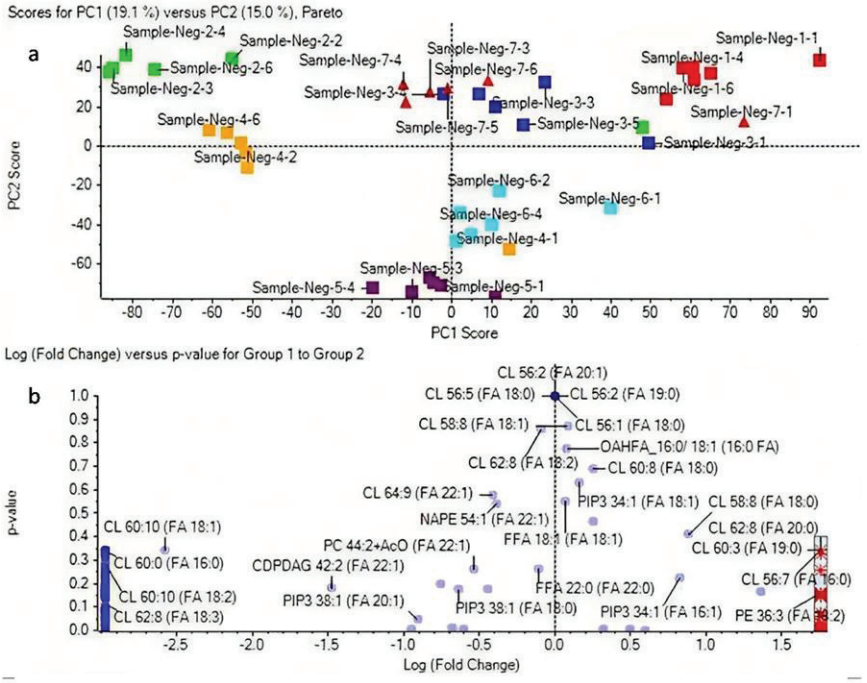
Principal component analysis

After lipid identification and relative quantitation, statistical multivariate analysis was done to understand the relative difference between lipids. Markerview™ Software was used to import the output of Lipidview™ software to visualize trends in lipid expression across seven different cultivated species of Brassica seed oil using principal component analysis (PCA).

Results and discussion

Lipid profile as detected in negative ion mode

A total of 70 lipid species belonging to different classes were detected in the negative ion mode. The percentage of variability explained by first two principal components is 34.1 %. In the Fig. 1a, samples 1, 2, 3, 4, 5, 6 and 7 represent *B. juncea*, yellow sarson, toria, brown sarson, *B. napus*, *B. carinata* and *Eruca sativa* respectively hereafter in this report. It shows the variation in the levels of these compounds among these species. When PC1 and PC2 were plotted



Sample-Neg-1.1 to Sample-Neg-1.6 (■) represent six replications of *Brassica juncea*. Likewise, Sample-Neg-2.1 to Sample-Neg-2.6 (■) correspond to six replications of Yellow Sarson. Sample-Neg-3.1 to Sample-Neg-3.6 (■) indicate Toria across replications 1 to 6. For Brown Sarson, Sample-Neg-4.1 to Sample-Neg-4.6 (■) represent replications 1 to 6. *Brassica napus* is denoted by Sample-Neg-5.1 to Sample-Neg-5.6 (■) across six replications. Similarly, Sample-Neg-6.1 to Sample-Neg-6.6 (■) correspond to six replications of *Brassica carinata*. Lastly, *Eruca sativa* is represented by Sample-Neg-7.1 to Sample-Neg-7.6 (▲) across replications 1 to 6.

Figure 1. a. PCA analysis showing the similarity among different species based on their lipid profile as detected in negative ion mode. **b.** Volcano plot representing MS data showing statistical significance and fold change for compounds detected in negative ion mode. Compounds on the right-hand side are over expressed and the ones on left-hand side are downregulated. CL: Cardiolipin, OAHFA: (O-acyl) ω-Hydroxy Fatty Acid, PIP: Phosphatidylinositolphosphate, NAPE: N-acylphosphatidylethanolamine, FFA: Free Fatty Acid, PC: Phosphatidylcholine, CDPDAG: Cytidine diphosphate diacylglycerol, PE: Phosphatidylethanolamine.

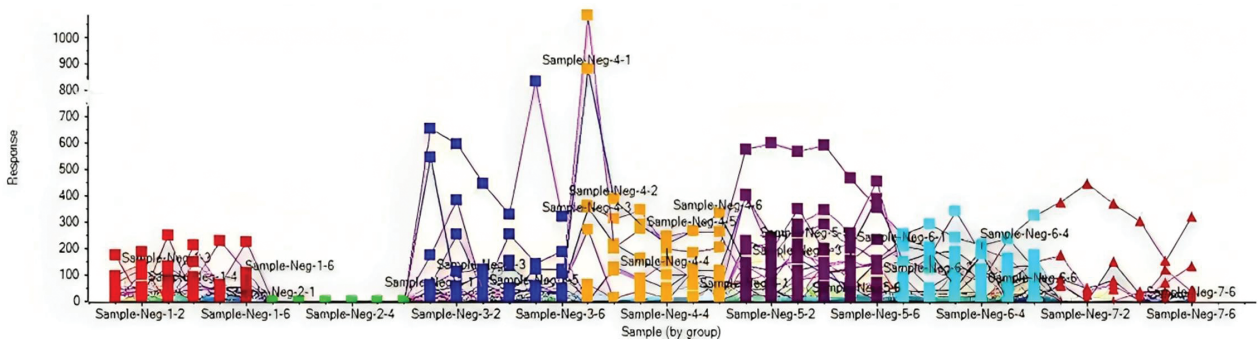


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Figure 2. Response of individual compounds detected in negative ion mode that are upregulated (right-hand side of the volcano plot).

against each other, *B. juncea* and toria showed considerable similarity in their spectrum (Fig. 1a). Yellow sarson, Brown sarson and *Eruca sativa* also appears to be similar in terms of MS spectrum. The volcano plot representing the MS data (Fig. 1b) showed the statistical significance and fold change. Points appearing away from the centre have large magnitude of fold change and those points having low p-value (<0.05) are statistically significant. Compounds satisfying these two conditions can be considered as differentially expressed among the seven species.

Fig. 2 shows the response of individual compounds such as CL 58:8 (FA 18:0), CL 62:8 (FA 20:0), CL 60:3 (FA 19:0), CL 56:7 (FA 16:0), Phosphatidylinositolphosphate (PIP) 3 34:1 (FA 16:1) and PE 36:3 (FA 16:2) that are present in higher levels (right side of the volcano plot) in each species. It also shows that in brown sarson and toria these individual compounds expressed a higher response compared to other species.

Free fatty acids

Edible oils with low saturated fatty acids are preferred as they are reported to cause increase in blood cholesterol levels proportional to the length of their carbon chains (Denke 2006). Monounsaturated fatty acids (MUFA) have been attributed with influencing blood lipids, blood pressure, insulin sensitivity and anti-obesity properties (Gillingham et al. 2011). In the present study, 8 species of FFAs were found to be in significantly higher levels including palmitic, stearic, oleic, linoleic, arachidic, Eicosanoic, behenic and erucic acids while linolenic acid was not detected (Fig. 3). Erucic acid was the most abundant FFA and showed exceptionally high levels in yellow and brown sarson species compared to others. This is followed by Palmitic and stearic acids which shows that saturated FFAs are more abundant in these oils compared to unsaturated FFAs. Linoleic acid was present in free form only in yellow and brown sarson species. Free eicosanoic acid was present only in yellow sarson and *Eruca sativa* while it was almost absent in other species. It is worthwhile to note that yellow sarson oil contain the highest levels of almost all FFAs compared to others.

Genotypes of *B. napus* that are low in erucic acid (<2 %) are known as canola (Ali et al. 2009). The major fatty acids reported in canola oil are Palmitic acid (C16:0), stearic acid (C18:0), cis-vaccenic acid (C18:1), oleic acid (C18:1), octadecanoic acid (C18:1), eicosanoic acid (C20:1), docosanoic acid (C20:1) and erucic acid (C22:1) (Schwender et al. 2015). It is known to contain low levels of saturated fatty acids (5–7 %) and high levels of polyunsaturated fatty acids (PUFA) with 7–10 % linolenic acid (ω-3) and 17–21 % linoleic acid (ω-6) (Pellet et al. 2008; El-Beltagi and Mohamed 2010). Teh and Birch (2013) analyzed canola oil by gas chromatography (GC) to find oleic acid to be the predominant fatty acid (57 %). Similar observations were made by El-Beltagi and Mohammed (2010) in *B. napus* by GC-MS. They have reported oleic acid to range from 56.31–58.67 % while erucic acid content between 0.15–0.91% qualifying them as canola type. However, not all *B. napus* varieties are low in erucic acid. 1H NMR study also shows *B. napus* to contain erucic acid content between 0 to 42 % and with increase in erucic content oleic acid level would decrease. In our study, there was considerable variation among the species as yellow sarson and *B. juncea* had higher levels of saturated FFA contrary to previous reports (Han et al. 2016).

Previous studies on various important oil crops have revealed distinct fatty acid profiles. Jokic et al. (2013) reported that soybean oil is primarily composed of linoleic acid (55.968%), with low levels of erucic acid (0.592%). Considerable level of lignoceric acid was also detected. In the case of Algerian peanuts, Giuffre et al. (2016) found that oleic acid dominates the fatty acid profile at 50.94%, with minimal levels of linolenic acid (0.19%) and palmitoleic acid (0.04%). Sunflower oil, as characterized by Rosa et al. (2009), is distinguished by high linoleic acid content (69.62%), the absence of myristic acid, and low levels of linolenic acid (0.02%). In the fatty acid profile of tomato seed oil, Giuffre et al. (2017) identified the presence of margaric acid (0.11%), palmitoleic acid (0.45%), and ginkgolic acid (0.39%). Notably, among these oils, erucic acid

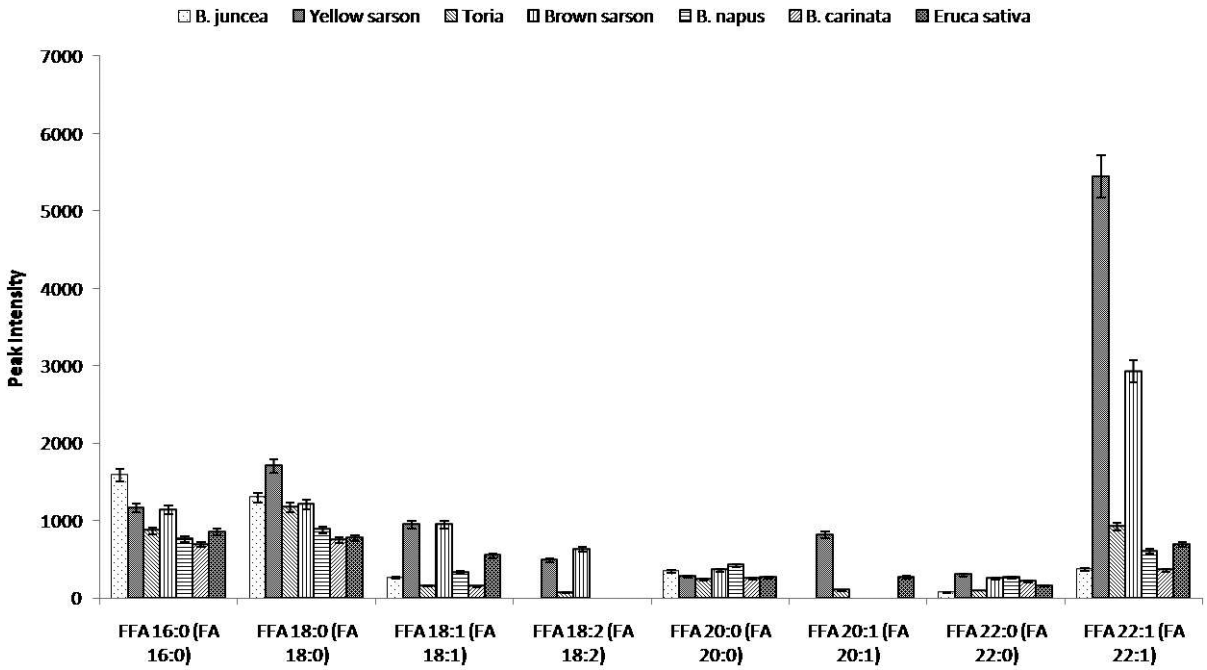

Figure 3. Variation in response of free fatty acids among different species based on their lipid profile as detected in negative ion mode.

was only detected in soybean oil, similar to its presence in mustard oil. Brassica seed oils, in contrast, lack detrimental saturated fatty acids such as myristic, margaric, and lignoceric acids. Additionally, arachidic and behenic acids are present in minimal concentrations in the free fatty acid form (Fig. 3). Nevertheless, the elevated levels of erucic acid in the composition could be deemed a disadvantage.

Eicosanoic acid levels have been reported to be 1.27 and 2.91 % for rapeseed and mustard oil respectively (Vyviurska et al. 2015). Higher levels of eicosanoic acid have been reported for mustard oil (6.89 %) and rapeseed oil (9.3 %) in other studies (Abul-Fadl et al. 2011). However we did not observe high erucic varieties to have high levels of eicosanoic acid as it has been described in a previous report (Richter et al. 2010). No correlation was observed between eicosanoic and erucic acid levels. Linolenic acid (ω-3) which is an important PUFA essential for brain development and cardiovascular diseases, usually found in a range of 6–14 % was not detected as FFA in any of the oils. In the GSL-1 variety of *B. napus* which we have analyzed, stearic acid was the most abundant FFA followed by palmitic and erucic acid. Since it is not canola type, it is expected to have high erucic acid and low oleic acid content. Exceptionally high level of erucic acid was observed in yellow sarson and brown sarson. FFA and total fatty acid content would be different and would have no correlation with each other. And also there are no reports showing yellow sarson to be so high in erucic acid content compared to other species. The PCA analysis reveals that *B. juncea*, *B. napus* and *B. carinata* have much similarity in their MS spectrum. FFA 22:1, FFA 20:0 and FFA 16:0 are showing significant variation and differential expression. Fig. 4a, b shows that FFA 16:0 (palmitic acid), FFA 16:1 (palmitoleic acid) and FFA 14:0 (myristic acid) were upregulated and their response was highest in *B. juncea*.

Cardiolipins

Cardiolipins are phospholipids present exclusively in the inner mitochondrial membranes where they are essential for regulating various kinds of mitochondrial proteins such as electron transport complexes, carrier proteins and phosphate kinases (Nakagawa 2013). They have four fatty acyl moieties that determine their diversity and are susceptible to peroxidation and contribute to membrane fluidity (Van Klompenburg et al. 1997).

Cardiolipins (CLs) play a crucial role in the respiratory chain, and variations in their fatty acid composition have been linked to various disorders, such as pulmonary hypertension, heart failure (Saini-Chohan et al. 2011), acute myocardial ischemia and reperfusion (Petrosillo et al. 2003) and diabetes mellitus (Han et al. 2007). Higher levels of circulating linoleic acid (LA) and muscle-derived tetralinoleoyl-cardiolipin (LA4CL) have been associated with a reduced risk of cardiometabolic diseases. LA-rich oil fortification has been shown to increase LA4CL, contributing to a lowered risk of such diseases (Cole et al. 2022). Although the role of CL and its metabolism in plants is not well understood, measuring CL in plants poses challenges due to its low abundance and the presence of interfering compounds like galactolipids, neutral lipids, and pigments. To overcome these challenges, solid-phase extraction via anion exchange chromatography was employed by Zhou et al. (2016) to purify CL from crude plant lipid extracts. They employed LC/MS analysis to reveal the content and molecular species composition of CL in various plant species, including Arabidopsis, mung bean, spinach, barley, and tobacco.

In our study, out of 26 different species of cardiolipins detected in negative ion mode, CL 56:1 (FA 18:1) and CL

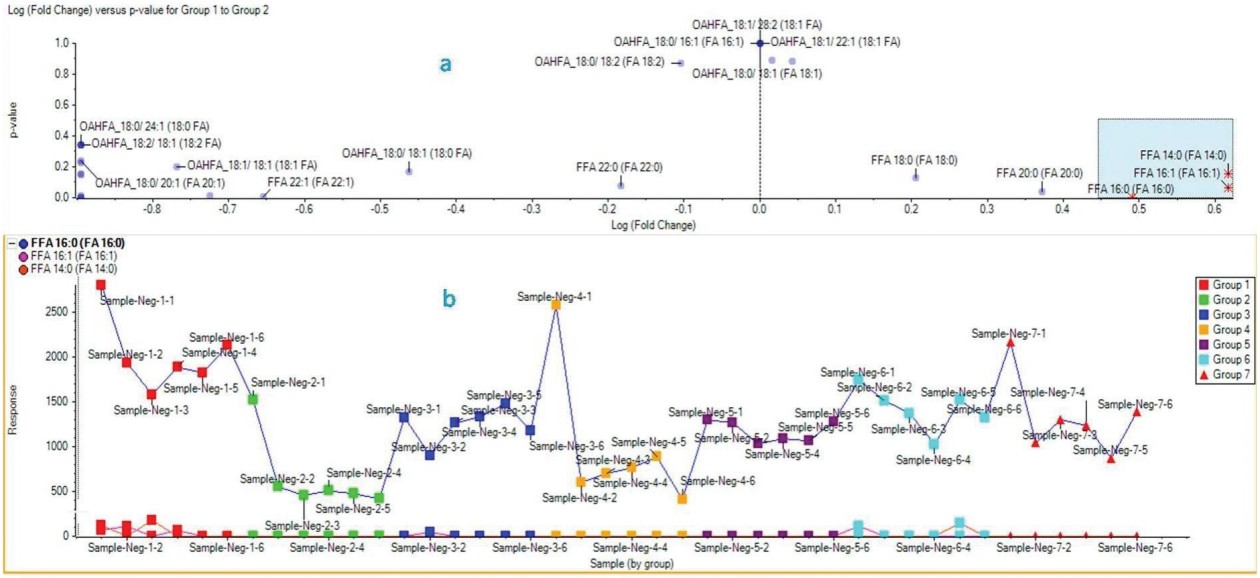

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Figure 4. a. Volcano plot representing MS data showing statistical significance and fold change for different fatty acids detected in negative ion mode. **b.** Response of different fatty acids that are upregulated (right-hand side of the volcano plot). OAHFA: (O-acyl) ω-Hydroxy Fatty Acid, FFA: Free Fatty Acid.

56:1 (FA 22:1) were present only in brown sarson. Similarly, CL 56:2 (FA 18:2) and CL 56:1 (FA 22:1) were present only in Yellow and Brown sarson. Rest of the cardiolipins was detected only in *B. napus* oil. CL 60:7 (FA 16:0) and CL 62:9 (FA 20:0) signals were obtained in *B. carinata*. CL 60:1 (FA 22:1) and CL 62:8 (FA 18:1) were absent in all other species.

N-Acyl phosphatidylethanolamines (NAPEs)

N-acylphosphatidylethanolamines (NAPEs) are nitrogen containing lipids belonging to glycerophospholipid class. NAPEs amount to 2–3 % of total phospholipid content in plants (Chapman et al. 1999). Diversity in NAPEs is due to the variation in length and number of double bonds in the acyl chains (Kilaru et al. 2012). N-acylethanolamines (NAEs) formed by hydrolysis of NAPEs have role in seed germination and seed establishment. Both NAPEs and NAEs have been detected more in desiccated seeds and their levels go down post germination indicating their role in plants (Chapman 2004). NAPEs are also known to have membrane stabilizing properties in both plants and animals. However, the main function of NAPE is as the precursor of NAEs which carries out lipid mediated functions in cells (Coulon et al. 2012). De Luca et al. (2019) utilized LC-HRMS to evaluate the levels of N-acylphosphatidylethanolamines (NAPEs), N-acylethanolamines (NAEs), and endocannabinoids (ECs) in 43 food products. They simulated daily intakes based on Mediterranean, vegetarian, and Western diets. The results revealed that plant-based foods exhibited higher abundance of NAPEs and NAEs compared to animal food products. Alves et al. (2021) employed ESI-MS and MS/MS spectra through a Q-ToF mass spectrometer in positive ion mode and/or a linear ion-trap mass spectrometer in both positive and negative ion modes. The MS spectra showcased molecular ions corresponding to lipid classes in olive seeds. NAPEs were identified in the negative ion mode as $[M-H]^-$ ions.

Data from the present study reveals four different forms of NAPEs including NAPE 52:1 (FA 22:1), NAPE 54:12 (FA 18:1), NAPE 56:12 (FA 22:1) and NAPE 58:12 (FA 22:1) in different samples. All four forms of NAPEs were present in yellow sarson while only NAPE 56:12 (FA 22:1) was present in brown sarson. Other Brassica species did not show presence of any of these NAPEs. Yellow sarson variety seems to be peculiar as it contains all four forms of NAPEs. Although, the specific functions of these NAPEs are not known, yellow sarson variety can be considered as distinct due to this property.

(O-acyl) ω-Hydroxy fatty acids (OAHFAs)

(O-acyl) ω-hydroxy fatty acids (OAHFAs) are polar lipids found in meibomian gland secretions in animals. Earlier OAHFAs have been reported in canines, rabbits, mice and humans (Butovich et al. 2012). OAHFAs function as a surfactant in the tear film lipid layer (Schuett and Millar 2013).

Wheat varieties with different wax content were selected to comparatively analyze their waxy components using ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) by Zheng et al. (2021). Through lipidomic analysis, 1287 lipid molecules were identified by them representing 31 lipid subclasses including OAHFA lipids. In our study, OAHFA 16:0/ 18:1 (16:0 FA), OAHFA 18:0/ 18:2 (FA 18:2), OAHFA 18:0/ 22:1 (FA 22:1) and OAHFA 18:0/ 18:1 (FA 18:1, 18:1 FA) were the four different forms of OAHFA detected (Suppl. material 1). All four forms were present in yellow sarson while OAHFA 16:0/ 18:1 (16:0 FA) was absent in brown sarson. None of the other species gave any MS signals for these compounds. According to the volcano plot, (Suppl. material 2) the compounds OAHFA 18:1/22:1 (FA 22:1), OAHFA 18:0/22:1 (FA 22:1), OAHFA 18:0/18:2 (18:0 FA), OAHFA 18:1/20:1 (FA 20:1) etc. were downregulated. Yet again, yellow sarson variety appears to be distinct in having all four forms of OAHFA making it nutritionally important. Since OAHFA has been rarely reported in plants, it is necessary to confirm their presence in rapeseed oil using suitable standards which we could not do due to unavailability of standards.

Phospholipids

Phospholipids are major constituents of biological membranes and are responsible for maintaining membrane integrity and cell homeostasis (Taguchi et al. 2005). Phospholipids are involved in cell growth and differentiation (Skwarek and Boulianne 2009). The main group of phospholipids is the glycerophospholipids that includes phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS). PIs also function as metabolic precursor for polyphosphoinositides that has role in signal transduction and cell cycle progression besides being an integral part of cell membranes (Horn and Chapman 2014). PSs contain amino groups and have antibiotic synergism with vit-E (Totani 1997). PIPs are phosphorylated forms of PIs that are collectively called as phosphoinositides and have roles in lipid signaling, cell signaling and membrane trafficking (Gillooly et al. 2001). In this study, MS response for 7 phosphatidylcholines (PC), 3 phosphatidylethanolamines (PE), 11 phosphatidyinositols (PI), 1 phosphatidylinositolphosphate (PIP), 3 phosphatidylinositol triphosphate (PIP 3), 1 phosphatidylserine (PS) and 1 cytidine diphosphate diacylglycerol (CDPDAG) were recorded. PC 36:2+AcO (FA 18:1) was the most abundant phospholipid and *B. napus* had the highest level. PI 34:2 (FA 16:0) and PI 34:2 (FA 18:2) were also present in high amount in brown sarson. CDPDAG 40:3 (FA 22:1) was detected only in *B. napus* oil. As we know that CDPDAGs are liponucleotides that are present in extremely small amounts in tissues (<0.05 % of total phospholipids) and hence very rarely reported. It is a precursor for many other phospholipids (Qi et al. 2016). PS 40:6 was detected only in brown sarson. Almost all forms of phospholipids were detected only in *B. napus* species while they were absent in others. Since *B. napus* is having almost all forms of phospholipids, they stand out as nutritionally important.

Lipid profile as detected in positive ion mode

In positive ion mode, a total of 1098 lipid species were detected including digalactosyldiacylglycerols (DGDG), sphingolipids, monoalkyldiacylglycerols (MADAG), N-acyl phosphatidylethanolamine (NAPEs) and phosphatidylcholines (PC). A total of 165 DGDGs, 474 triacylglycerols (TAG), 12 phosphatidylcholines, 315 NAPEs, 45 MADAGs, 1 MGDG and 86 different species of sphingolipids were detected. According to PCA results (Suppl. material 3), yellow sarson, toria and brown sarson have much similarity in their spectrum. *B. napus* and *Eruca sativa* also appears to have similarity in their components. However, *B. juncea* and *B. carinata* stands out to be unique. The percentage of variability explained by first two principal components is 63.8 %. However, maximum variability according to the volcano plot (Suppl. material 4) was observed in sphingolipid species such as GT2 42:3:2, GM3 26:2:2, GT3 44:3:4, GT3 46:1:2 that were upregulated. Considerable variation is also seen in the response of individual compounds among different species. At the same time, compounds like GTI 26:0:3, GTI 30:0:3, GDI 42:1:2, GDI 42:1:2 etc. were downregulated and their response was most prominent in yellow sarson (Suppl. material 3).

Sphingolipids

Sphingolipids (SLs) have a sphingoid base backbone, composed of sphingosine (So), sphinganine (Sa), or 4-hydroxysphinganine (phytosphingosine). Ceramide (Cer) is formed when a fatty acid (FA) is attached to carbon-2 (C-2) on these backbones via an amide bond. Subsequently, complex SLs are produced by attaching hydrophilic head groups to the OH-group at C-1 (Futerman and Riezman 2005). The distribution and quantity of dietary SLs vary widely across different foods, with low content in fruits and vegetables and high content in dairy products and soybeans (Vesper et al. 1999). Dietary SLs can promote the elimination of pathogenic organisms and toxins from the intestine (Duan and Nilsson 2009), influence viral receptors (Utermohlen et al. 2008), regulate cell fate determination, cancer initiation, progression, and drug sensitivity (Newton et al. 2015), play a role in multiple signaling pathways governing neuronal development (Piccinini et al. 2010), help in skin hydration (Lee et al. 2015), contribute to adipose tissue function and treating obesity (Le Barz et al. 2020).

Ines et al. (2018) had quantified the total sphingolipids by the analysis of their released long-chain bases (LCB) using reverse-phase HPLC. Hu et al. (2021) established and applied an ultrahigh-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry method for a comprehensive lipid-

omic profiling of oilseeds. Their study identified and quantified 15 sphingomyelins along with other major lipid species.

The study identified a total of 86 different forms of sphingolipids. These included 6 GD1, 4 GM3, 32 GT1, 25 GT2, 4 GT3, 10 Hex3Cer, and 4 MIPC types. Separate PCA and volcano plots were generated for the sphingolipids. Significant variations were observed in GD1 42:1;2 (LCB 18:0;2-2H2O) and GT3 44:1;4 (LCB 18:0;2-2H2O). Each species exhibited distinct distributions of sphingolipids.

Phosphatidylcholines (PC)

Phosphatidylcholines constitute a major group that has structural and functional role in cell membranes. It also has pharmaceutical applications in treatment of neurological and liver diseases (Reddy et al. 2005). Up on studying the effect of PC and PE concentration on the oxidation rate of stripped peanut oil (SPO) and bulk peanut oil (BPO) with Electron paramagnetic resonance (EPR), it was found that PC and PE decelerated BPO oxidation (Zhao et al. 2020). On the contrary increasing PC concentrations (50–1000 ppm) had substantial reductions in antioxidant efficacy (AE) of Trolox. The effectiveness of both α-tocopherol and trolox decreased significantly in the presence of PC. (Velasco et al. 2023).

Thin layer chromatography (TLC) has been employed by Yang et al. (2020) to purify PCs from six different beans, followed by their identification through ultra-high-performance liquid chromatography–Quadrupole (Q)–high-resolution mass spectrometry (UHPLC-Q-HRMS). The findings revealed that chickpea (*Cicer arietinum*) and soybean (*Glycine max*) exhibited PC contents of 50.0 and 34.0 mg/g, respectively, which is higher than the levels observed in the other beans. Zitouni et al. (2016) had utilized nanospray ionization quadrupole time-of-flight mass spectrometry to analyze glycerophospholipid classes and molecular species in the seed oils of two halophytes, *Cakile maritima* and *Eryngium maritimum*. Phosphatidylcholine emerged as the predominant glycerophospholipid in both oils, with phosphatidylethanolamine and phosphatidic acid being less abundant. Quantitative variations were observed in the main molecular species (C36:4, C36:3, C36:2, 34:2, and C34:1) among the different glycerophospholipids and between the two halophytes.

PC 48:0 (LPC) was the most abundant phosphatidylcholine detected in this study followed by PC 48:1 and PC 48:2. *Eruca sativa* showed highest level of PC 48:0 (LPC) followed by *B. napus*. PC 44:1 was detected only in *B. napus* and *Eruca sativa* oils. PC 45:6 was detected only in *Eruca sativa* and PC 48:4 was present only in *B. juncea* and *B. carinata*.

Conclusions

Major fatty acids, phospholipids, sphingolipids and storage lipids could be identified and their relative abundance was compared among the species. As ESI source is known to work best for detecting polar lipids, some of the non polar lipids may have been undetected. Hence, it is necessary to perform MS analysis again using an APCI source. Almost all forms of cardiolipins were present in *B. napus* while most of them were absent in other species. Saturated FFAs were lowest in *B. carinata*. Important phospholipids such as cardiolipins and NAPEs and their relative abundance among the different species could be understood. OAHFAs which have been reported only in animal system have been detected in Brassica seed oil which needs further confirmation. As high levels of saturated FFAs are not desirable, *B. carinata* can be considered beneficial due to their low levels. Yellow sarson variety contains all four forms of NAPEs and all four forms of OAHFA. Yellow along with brown sarson were having highest levels of erucic acid in the form of FFA. These properties make them nutritionally important. Also, since *B. napus* is having almost all forms of phospholipids and cardiolipins, it stands out as nutritionally important.

Authors contribution

SK: design of experiment, laboratory work, writing; AS: data analysis, editing; FR: laboratory work, data analysis, writing, editing; IM: laboratory work, writing, editing. RR: literature, proof reading, editing; VVS: literature, editing; BM: proof reading, editing; MP: proof reading, editing.

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Supplementary material

Supplementary material 1
Supplementary fig. S1
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Supplementary material 2
Supplementary fig. S2
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Supplementary material 3
Supplementary fig. S3
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Supplementary material 4
Supplementary fig. S4
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