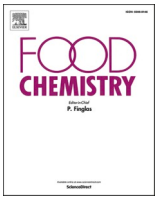
Food Chemistry 454 (2024) 139790

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Comprehensive analysis of oxidative stability and nutritional values of germinated linseed and sunflower seed oil

Tobias Pointner [a,b], Katharina Rauh [a], Arturo Auñon-Lopez [a,b], Sanja Kostadinović Veličkovska [c], Saša Mitrev [c], Emilija Arsov [c], Marc Pignitter [a,*]

[a] Institute of Physiological Chemistry, Faculty of Chemistry, University of Vienna, 1090 Vienna, Austria
[b] Vienna Doctoral School in Chemistry (DoSChem), University of Vienna, Währinger Str. 42, 1090 Vienna, Austria
[c] Faculty of Agriculture, University Goce Delčev, Štip, Republic of North Macedonia

ARTICLE INFO

Keywords:
Germinated oils
Oxidative stability
Antioxidant capacity
Polyphenols
Shelf-life

ABSTRACT

Germination of seeds is known to affect the nutritional composition of cold-pressed oils. This study focused on the effects of germination on the antioxidants and oxidative stability of linseed and sunflower seed oil. As hypothesized, germination led to increased antioxidant activities and tocopherol, chlorophyll and carotenoid content. Analysis revealed a 37.2 ± 3.5-fold and 11.6 ± 1.5-fold increase in polyphenol content in linseed and sunflower seed oil from germinated seeds, respectively. Using LC-HRMS/MS, profiles with up to 69 polyphenolic substances were identified in germinated seed oils for the first time. Germination promoted lipid hydrolysis, as evidenced by NMR, with overall significant decreases in triacylglycerol content leading to increased diacylglycerol and free fatty acid values. Rancimat measurements predicted a 4.10 ± 0.52-fold longer shelf-life for germinated linseed oil. This study successfully demonstrated the potential of germination to develop PUFA-rich oils with enhanced antioxidant capacity and oxidative stability.

1. Introduction

Linseeds and sunflower seeds have proven to be not only a staple food but also a significant source of bioactive compounds with potential health benefits, making them interesting for both consumers and manufacturers in further processing. Linseed (*Linum usitatissimum L.*) possesses a nutritional profile rich in essential fatty acids, vitamins, minerals, fiber, and proteins. Approximately 45% of the seed is oil, 30% is fiber, and 25% is protein, demonstrating the versatility of these seeds (Dixit, Kendurkar, Vajpeyi, & Prasad, 2007). Sunflower seeds (*Helianthus annus* L.), which are primarily used for the production of sunflower oil, have an impressive 58% lipid and 15% protein content, as well as a high amount of 18 different essential minerals (Petraru, Ursachi, & Amariei, 2021; Prado et al., 2020).

Cold-pressed linseed oil, mainly composed of the polyunsaturated fatty acid (PUFA) α-linolenic acid (ALA), is noteworthy for its contribution to a balanced omega-6 to omega-3 ratio in the diet (Farag, Elimam, & Afifi, 2021). Sunflower oil, primarily composed of linoleic and oleic acids, represents an important source of energy (A. Premnath, Narayana, Ramakrishnan, Kuppusamy, & Chockalingam, 2016; Khan, Choudhary, Pandey, Khan, & Thomas, 2015). In addition to their fatty acids, these oils contain various bioactive substances, such as tocopherols and phenolic compounds, which further improve their nutrient richness and quality (Yang et al., 2021). Moreover, these antioxidants positively contribute to storage stability and potential health benefits (Li et al., 2018; Nadeem, Anjum, Khan, Saeed, & Riaz, 2011). The bioactive properties of linseed oil attributed to ALA and the phenolic compound secoisolariciresinol highlight its potential health benefits, including cardiovascular, antioxidant, and anticancer properties (Singh, Mridula, Rehal, & Barnwal, 2011; Touré & Xueming, 2010). The polyphenolic spectrum of sunflower seed oil, including chlorogenic acid, caffeic acid, and p-hydroxybenzoic acid, emphasizes the complexity and richness of this oil as well (Leung, Fenton, & Clandinin, 1981).

However, the susceptibility of unsaturated oils, particularly those rich in polyunsaturated fatty acids, to autoxidation is a critical concern in the food industry (Pignitter, Grosshagauer, & Somoza, 2019;

* Corresponding author.
E-mail addresses: tobias.pointner@univie.ac.at (T. Pointner), a01448537@unet.univie.ac.at (K. Rauh), arturo.aunon-lopez@univie.ac.at (A. Auñon-Lopez), sanja.kostadinovik@ugd.edu.mk (S. Kostadinović Veličkovska), sasa.mitrev@ugd.edu.mk (S. Mitrev), emilija.arsov@ugd.edu.mk (E. Arsov), marc.pignitter@univie.ac.at (M. Pignitter).

https://doi.org/10.1016/j.foodchem.2024.139790
Received 29 March 2024; Received in revised form 13 May 2024; Accepted 20 May 2024
Available online 22 May 2024

Yamamura et al., 1995). Linseed oil, in particular, with its high ALA content, poses a major challenge owing to lipid oxidation, which results in a relatively short shelf-life. The oxidative stability of these oils depends on a complex interplay of factors including fatty acid composition and antioxidant activity (Kozłowska, Gruczyńska, Ścibisz, & Rudzińska, 2016; Parker, Adams, Zhou, Harris, & Yu, 2003; Prescha, Grajzer, Dedyk, & Grajeta, 2014; Turgut Dunford, 2015).

Recently, seed germination has been proposed as a cost-effective and sustainable pretreatment process to enhance the nutritional content and quality of oils (Cáceres, Peñas, Martinez-Villaluenga, Amigo, & Frias, 2017; Rico et al., 2020). Germination activates a metabolic cascade driven by hydrolysis and energy production (Renu Joshi, 2018), which results in the breakdown of the endosperm and seed coat, thereby releasing bioactive compounds with potential health benefits (María Landete et al., 2015; Pająk, Socha, Gałkowska, Rożnowski, & Fortuna, 2014; Somsong, Santivarangkna, Tiyayon, Hsieh, & Srichamnong, 2020; Wang et al., 2016). Studies have shown that germination increases the content of essential nutrients such as vitamins, minerals, and their concomitants (Herchi et al., 2014; Li et al., 2019; Zhang et al., 2023). Moreover, germination has the potential to increase the carotenoid and chlorophyll contents of oils (Herchi et al., 2015). However, it is still unknown whether the germination process influences the formation of antioxidants, particularly specific phenolic substances, which may also be enriched in cold-pressed oil. In addition, to date, there are no data available that describe lipid oxidation in oils from germinated seeds in detail and, subsequently, their oxidative stability and shelf-life.

Our research was based on the hypothesis that the germination pretreatment of linseeds and sunflower seeds could have positive effects on the nutritional value, oxidative stability and shelf-life of cold-pressed oils. Through a detailed analysis of conventional oils and those from germinated seeds, we aimed to address the existing knowledge gaps and provide valuable insights into the antioxidative properties, polyphenol profiles, and oxidative stability of these oils.

2. Materials and methods

2.1. Chemicals and materials

All chemicals were purchased from reputable suppliers such as Sigma-Aldrich (Vienna, Austria), Carl Roth (Karlsruhe, Germany), and VWR International GmbH (Vienna, Austria). The solvents used for chromatography were of LC-MS grade quality.

2.2. Seeds and oil samples

Linseeds (*Linum usitatissimum L.*) and sunflower seeds (*Helianthus annuus L.*) were sourced from the Republic of North Macedonia and obtained from the company Agrofila, Stip. Cold-pressing of the seeds was performed in a hermetically sealed system below 60 °C using a screw extruder to preserve the integrity of the oils and minimize heat-induced degradation. The oil yield was determined based on the volume of oil pressed per kg of seed before and after germination. Oil samples were stored at −80 °C until further sample preparation and analysis.

2.3. Germination process

The germination process started with seed washing (H_2O), followed by disinfection in a 2% sodium hypochlorite (NaOCl) solution by shaking for 2 min (Sauer & Burroughs, 1986). After rinsing with sterile water, the seeds were placed on wet filter paper in plastic containers and placed in a dark chamber for four days at 25 °C and constant humidity, ensuring a residual water content of 20%-30% (Guo, Klinkesorn, Lorjaroenphon, Ge, & Na Yom, 2021). Overall, the germination process took five days in an incubator. This period was selected based on established practices in seed germination studies and previous research demonstrating that this duration allows for optimal biochemical changes as lipid and protein composition in the seeds while maintaining seed viability (Herchi et al., 2015; Zhang et al., 2023). After germination, the seeds were dried in an oven at 50 °C for 21 d (linseed) and 25 d (sunflower) until the residual water content was below 4% (Dong et al., 2024; Huang et al., 2024). A visual comparison of the oil samples can be seen in Fig. S1.

2.4. Determination of fatty acid profiles by gas chromatography-flame ionization detection (GC-FID)

Fatty acids were analyzed as their respective fatty acid methyl esters (FAME) via GC-FID (GC-2010 Plus, Shimadzu, Korneuburg, Austria). For the extraction and hydrolysis of fatty acids from the oil samples and their subsequent analysis, a well-established FAME method was employed, based on Grüneis et al., 2019. The sample preparation process followed the protocols of Lall, Proctor, & Jain, 2009 and Pignitter et al., 2014 with some modifications.

In brief, 100 mg of oil was combined with 1% heptadecanoic acid methyl ester (HME) internal standard solution, toluene, pyrogallol, and 0.5 M sodium methoxide. After incubation of the mixture under Ar atmosphere and quenching with acetic acid, liquid-liquid extraction was performed with n-hexane. The upper organic phase was collected, dried with sodium sulfate, and filtered through a 0.45 µm PVDF filter. Standards were prepared using a stock solution of methyl palmitate. All samples were analyzed using GC-FID.

2.5. Cyanogenic glycosides

The ÖNORM EN 16160, 2010 standard HPLC method was employed for the quantification of both bound and free cyanides in animal feed and raw materials of herbal origin at the accredited laboratory Institute of Animal Nutrition and Feed, Vienna. This method has been validated within the range of 10 to 350 mg HCN/kg with a limit of detection (LOD) of 1 mg/kg and limit of quantification (LOQ) of 3 mg/kg.

2.6. Antioxidants

2.6.1. Extraction of phenolic fractions and antioxidant capacities

To extract phenolic compounds from the oil samples, 2.5 g of oil was dissolved in 2.5 mL hexane (Singleton, Orthofer, & Lamuela-Raventós, 1999). Subsequently, 1 mL methanol/H_2O (80:20, *v*/v) was added to the solution. After vortexing for 4 min, the samples were centrifuged (Eppendorf® Centrifuge 5810R) at 2880 ×*g* at 4 °C for 5 min, and the polar phase was collected. This extraction and centrifugation process was repeated three times. The collected 3 mL extract was washed with 2.5 mL n-hexane (30 s vortexing; 6 min, 2280 ×*g*, 4 °C centrifugation), and the organic phase was discarded.

To determine the free radical scavenging capacity, two common assays, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH), were used. ABTS (7 mM) and potassium persulfate (2.5 mM) solutions were mixed and incubated overnight (Re et al., 1999). Afterwards, DPPH reagent (4.9 mg in 50 mL methanol) was prepared. The DPPH assay involved incubating 1 mL of reagent with 1 mL of sample extract, blank (methanol 80%), or standard for 1 h in the dark at room temperature. Standards were prepared using a Trolox stock solutions (Brand-Williams, Cuvelier, & Berset, 1995). ABTS and DPPH assay measurements were conducted at 734 nm and 517 nm, respectively, and expressed as µmol Trolox equivalent (TE)/kg.

The ferric reducing antioxidant potential (FRAP) assay was additionally performed, using a mix of buffer (1.5 g sodium acetate trihydrate and 8 mL acetic acid in 500 mL), 2,4,6-Tris(2-pyridyl)-*s*-triazine (33 mg TPTZ in 10 mL of 40 mM HCl), and $FeCl_3$ solution (20 mM) as FRAP reagent. Standards were prepared using an $FeSO_4 \times 7H_2O$ stock solution (Benzie & Strain, 1996). Measurements were conducted at 593 nm using a Spark photometer (Tecan Group Ltd., Switzerland).

2.6.2. Total polyphenol content

The total phenolic content (TPC) was determined in the phenols extracted from the oil samples (see section 2.6.1), using the Folin-Ciocalteu assay (Singleton & Rossi, 1965). The standards were prepared using a gallic acid solution. TPC was measured at 765 nm, and the results were expressed as mg gallic acid equivalent per kilogram (mg GAE/kg) of oil.

2.6.3. Polyphenols, phytochemicals, plastochromanol-8 and cyanogenic glycosides detection via liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS)

LC-HRMS/MS analysis, adapted from Fruehwirth et al., 2020, was performed to detect and characterize specific polyphenols enriched in the oil matrix due to the germination process.

In brief, polyphenol extraction involved dissolving 3.5 g of oil in 3.5 mL hexane and following the procedure (see 2.6.1). The methanol phase underwent N_2 evaporation and the aqueous phase was freeze-dried. The extracted polyphenolic samples were dissolved in 100 µL 80% methanol prior to injection. Plastochromanol-8 analysis included dissolving 0.05 g of oil in 1 mL 2-propanol and filtering through a PVDF filter (0.22 µm). A Vanquish Horizon UHPLC system (Thermo Fisher Scientific, Germering, Germany) system with a C18 column, coupled to a timsTOF fleX mass spectrometer (Bruker Daltonics), was used. Acetonitrile with 0.1% formic acid (A) and H_2O with 0.1% formic acid (B) constituted the mobile phase. The HPLC gradient ranged from 10% to 100% B over 21 min at a flow rate of 0.3 mL/min. The LC-HRMS/MS analysis was conducted using an electrospray ionization (ESI) source in positive and negative ion polarity modes, with a nebulizer pressure of 2.2 bar and a capillary voltage set to 4200 V. The RF collision cell was maintained at 1500 Vpp to facilitate analyte fragmentation. MZmine3.4.16 was used for data analysis (Schmid et al., 2023). The workflow included initial mass detection to generate a list of masses, followed by chromatogram generation, peak detection using MS^2 scans and subsequent filtering to remove noise. The masses of the detected substances are presented in Table S1.

2.6.4. Quantification of polyphenolic substances by targeted LC-MS/MS

Polyphenols were quantified using targeted LC-MS/MS. Polyphenolic substances were divided into the classes flavanols, flavones, flavonoids, lignans, phenolic acids, phenolic aldehydes, and stilbenoids. Representative standards from each of these classes, namely quercetin-3-glucoside, secoisolariciresinol, caffeic acid, ferulic acid, gallic acid, and resveratrol, were used to quantify all substances found. They were individually dissolved in 80% methanol/20% water at different concentrations and analyzed using an LC-MS system (LCMS-8040, Shimadzu, Korneuburg, Austria). The standard curves used to quantify individual polyphenolic substances are shown in Fig. S2. The LOD and LOQ for the specific phenolic substances were determined using the signal-to-noise ratio (S/N), where LOD and LOQ corresponded to S/N values of 3 and 10, respectively. These values were calculated based on the background noise in the chromatograms. Separation was performed on a C18 column (Kinetex EVO, 150 × 3.0 mm, 5 µm, Phenomenex, Aschaffenburg, Germany). The chromatographic conditions were the same as those for the untargeted LC-HRMS/MS analysis, maintaining a flow rate of 0.3 mL/min and an initial solvent B concentration of 10%. The HPLC system was coupled to a triple quadrupole MS with an ESI source operating in negative and positive multiple reaction monitoring (MRM) mode. The nebulizing gas flow and drying gas flow were set to 3 L/min (N_2) and 10 L/min (N_2), respectively, while the desolvation line (DL) temperature was maintained at 150 °C and the heater block temperature was 350 °C. Using Skyline v23.0 software, the LC-MS/MS data sets were processed using peak picking, alignment and quantification to characterize the specific polyphenols (Pino et al., 2017). The MRM transitions and the corresponding phenolic substances are shown in Table S2.

2.6.5. Tocopherols by HPLC/UV

For the determination of tocopherols, the method outlined by Gliszczynska-Swiglo, Sikorska, Khmelinskii, & Sikorski, 2007 was employed with slight modifications by Pignitter et al., 2014. Briefly, sample preparation involved placing 50 mg of the sample in a 1.5 mL Eppendorf tube and adding 1 mL 2-propanol. A rac-tocol dilution was prepared by mixing 100 µL of rac-tocol (50 mg/mL) with 900 µL of n-hexane. A total of 1 µL of the rac-tocol dilution was pipetted into each sample and the α-, γ-, and δ-tocopherol standards. After vortexing and filtration using PVDF filters, the samples were transferred into HPLC vials. Chromatographic analysis was performed on a Phenomenex Kinetex 5 µm EVO C18 column (150 × 4.6 mm), at 295 nm using a diode array detector (Dionex DAD-3000), enabling α-, γ-, and δ-tocopherol quantification and, consequently, vitamin E content determination. The standard curves are shown in Fig. S3.

2.6.6. Chlorophyll and carotenoids

For the analysis of chlorophyll and carotenoids, the method described by Herchi et al., 2015 was adopted. Sample preparation involved weighing 1.5 g of the sample in a 15 mL tube, followed by the addition of 5 mL cyclohexane. After vortexing and subsequent centrifugation at 2280 ×*g* and 4 °C for 6 min, the chlorophyll and carotenoid contents were measured at 670 nm and 470 nm, respectively, using a Spark photometer (Tecan Group Ltd., Switzerland).

2.7. Oxidative stability and shelf-life prediction

2.7.1. Peroxide and acid value determination

The peroxide value, indicative of the primary oxidation products, was determined using titrimetric analysis (Wheeler, 1932). For the determination, 5 g of oil sample was weighed into Erlenmeyer flasks. Then, 30 mL of acetic acid-chloroform (3:2) was added, followed by the addition of 0.5 mL of a potassium iodide 769.2 mg/mL solution. Subsequently, 30 mL of bi-distilled water and a small amount of starch were added. The upper phase of the solution turned brown in color. Titration was carried out with 0.1 N sodium thiosulfate solution until the upper phase was completely decolorized.

To determine the acid value, 3–5 g of the sample was weighed into a 250 mL Erlenmeyer flask (AOCS, 2017). The samples were dissolved in 100 mL of ethanol/diethyl ether (50:50) and heated for 45 s at 40 °C. Next, seven drops of phenolphthalein were added, followed by titration with 0.05 N potassium hydroxide (KOH) (diluted with ethanol 96%) until color change occurred. This procedure provided the acid value of the sample, expressed as mg KOH/g, indicating the amount of acidic substances present.

2.7.2. Analysis of acylglycerol composition by proton nuclear magnetic resonance (^1H NMR)

The ^1H NMR spectra of the samples were acquired using an Avance 400 spectrometer (Bruker, Vienna, Austria) operating at 400 MHz. Briefly, 100 µL of the oil sample was dissolved in 500 µL deuterated chloroform containing 0.2% non-deuterated chloroform and tetramethylsilane (0.03%) as an internal reference, allowing direct comparison between samples. The acquisition parameters used were adapted from Martínez-Yusta & Guillén, 2014: spectral width 6410 Hz, relaxation delay 3 s, 128 scans, 4.819 s acquisition time, and 90° pulse width. The resulting spectra were plotted and analyzed using MestReNova 12.0 (Mestrelab Research, Santiago de Compostela, Spain). The identification and quantification of glycerol structures in molar percentages were carried out based on previous studies (Alberdi-Cedeño, Ibargoitia, & Guillén, 2020; Nieva-Echevarría, Goicoechea, Manzanos, & Guillén, 2017), as the proportionality between the signals area and the number of protons that generate them render the analysis suitable for comparative purposes. The signal assignments and formulas for their determination are shown in Table S3 and Table S4, respectively. In addition, diacylglycerols content was determined by measuring a standard curve with

diolein, which is shown in Fig. S4.

2.7.3. Rancimat analysis

The oxidative stability of the oil samples was determined using the Rancimat method, following the protocols outlined by Aktar & Adal, 2019 and AOCS, 1995. This method allows for the evaluation of stability by measuring the oxidation induction time (OIT, in hours) with a Rancimat apparatus (Metrohm 892, Herisau, Switzerland). In all analyses, Rancimat vessels (Ø 24 × 150 mm) containing 3 g of oil samples were used, and an air rate of 20 L/h was maintained. The OIT of the linseed oil samples was assessed at temperatures of 100, 110, and 120 °C (Mène-Saffrané, Jones, & DellaPenna, 2010). The obtained data were utilized for shelf-life prediction using the Arrhenius equation.

$$ln(k) = ln\,A - \frac{E_a}{RT} \quad (1)$$

where A represents the pre-exponential factor (frequency factor), E_a stands for the activation energy in kJ/mol, R is the gas constant in kJ/(mol K) and T is the time-dependant temperature in K.

2.7.4. Secondary lipid oxidation products by solid phase micro extraction followed by gas chromatography-mass spectrometry (SPME/GC–MS)

Secondary lipid oxidation products were analyzed following a modified protocol described by Holler et al., 2023. In brief, 2.5 g of oil was placed in a 20 mL glass vial, and volatile compounds were extracted using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coated SPME fiber. The extracted compounds were analyzed using a GC–MS system (GC-QP2010 Ultra, Shimadzu, Korneuburg, Austria) equipped with a Zebron ZB-5 ms column. The identification was based on mass spectral matching using a commercial library (NIST, ver. 11.0), and semi-quantification was performed using arbitrary units of base peak ion area counts. The analyses were conducted in quadruplicate to ensure reliability.

2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism 9.4.1. The data were checked for normality using the Shapiro-Wilk test and for equality of variances using Levene's test before conducting ANOVA analysis. One-way analysis of variance (ANOVA) with Tukey's post-hoc test and unpaired *t*-test with control for multiple comparisons using the false discovery rate (FDR) approach by Benjamini, Krieger, and Yekutieli were conducted. The results were considered statistically significant different at a *p*-value <0.05. Each experiment was conducted with a sample size of $n \geq 3$.

3. Results & discussion

3.1. Effect of seed germination on the oil yield and the fatty acid profile of linseed and sunflower seed oils

The oil yield of the pressed sunflower seeds and linseeds was assessed before and after germination. Before germination, the oil content of sunflower seeds was 42.22 ± 0.24%. After germination, the oil content decreased significantly to an average value of 27.71 ± 0.21%. Similarly, the oil content of linseed before germination was slightly lower at 35.14 ± 0.45% and decreased to a value of 25.35 ± 0.3% after germination. There was thus a significant mass loss after germination in both sunflower seeds and linseeds, which could indicate metabolic processes such as respiration of the seeds or the utilisation of stored nutrients for the growth of the seedlings. These results highlight the influence of germination on oil content and mass loss in sunflower seeds and linseeds. The fatty acid profiles of the conventional and germinated linseed oil and sunflower seed oil were analyzed using GC-FID. A comparison of the oils revealed significant differences (Fig. 1), with linseed oil being characterized by higher levels of α-linolenic acid and sunflower seed oil exhibiting a predominance of oleic acid (Belo, Tognetti, Benech-Arnold, & Izquierdo, 2014). The observed variations in fatty acid composition were expected, as they were consistent with the established ranges for these oils (Deutsche Gesellschaft für Fettwissenschaft (Ed.), 2020).

The predominant fatty acid in conventional linseed oil was α-linolenic acid, which comprised 56% of the total fatty acids. Upon germination, minor fluctuations were observed in fatty acid composition. However, these changes were not statistically significant and reconfirmed previous findings by Herchi et al., 2015, who analyzed the fatty acid profile of oils over four days of germination. In contrast, the fatty

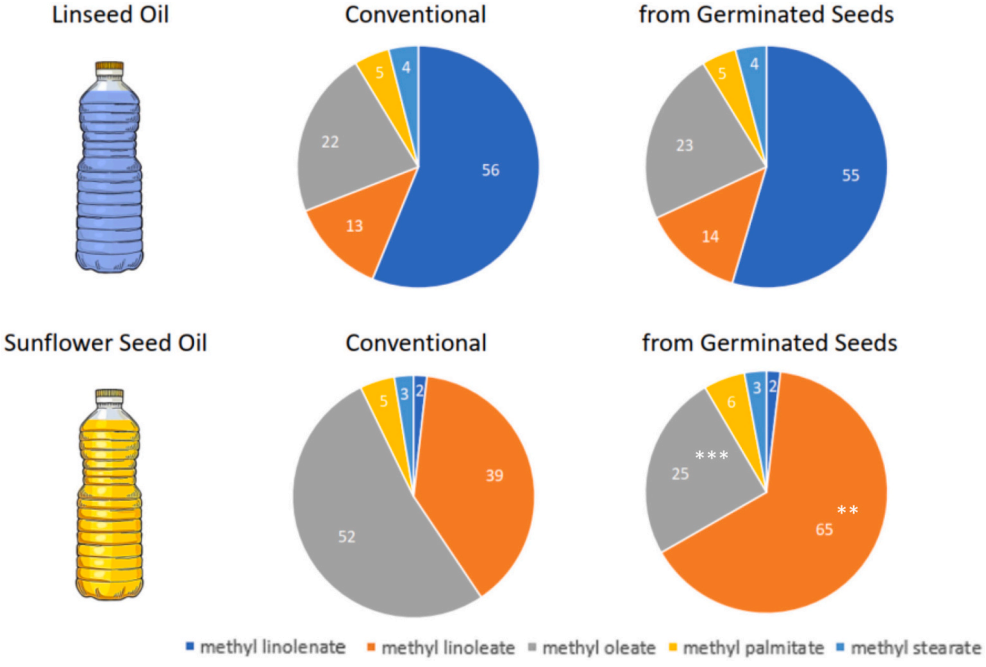


Fig. 1. Fatty acid profiles measured by GC-FID shows the fatty acid distribution of the oils in α-linolenic acid, linoleic acid, oleic acid, palmitic acid and stearic acid. One-way ANOVA was conducted with Tukey post-hoc test, and data are presented as mean, $n = 3$, ** = $p < 0.01$, *** = $p < 0.001$.

acid composition of conventional sunflower seed oil was characterized by a higher proportion of oleic acid (52%) followed by linoleic acid (39%). Upon germination, significant changes were observed, with a marked increase in linoleic acid content (up to 65%) and a corresponding decrease in oleic acid content (up to 25%). This shift in fatty acid composition suggests a metabolic response during germination, which can be explained on the one hand by the mobilization of stored reserves, such as triacylglycerols, to provide energy and building blocks for new cellular structures (Bewley, Bradford, Hilhorst, & Nonogaki, 2013). The elevated linoleic acid content in sunflower seed oil from germinated seeds reflect the preferential use of oleic acid for energy production or as a substrate for the synthesis of other compounds during germination. Since the linoleic/oleic acid proportion in linseed oil is already notably pronounced, there is no shift in the fatty acid profile. However, complex enzymatic processes are involved in germination (including enzymes such as lipases and desaturases), which regulate the breakdown and synthesis of various cellular components and thus bring about changes in the fatty acid composition of sunflower seed oil (Eastmond & Graham, 2001). To date, the fatty acid profile of oils from germinated sunflower seeds has not been investigated in detail. Therefore, further investigations are needed to elucidate the mechanisms underlying these changes.

3.2. Cyanogenic glycoside level increase in germinated oils

In general, HCN levels exceeding 50 mg/kg are considered hazardous to human health (Commisson Regulation (EU) 2022/1364, 2022). The standard reference HPLC method (ÖNORM EN 16160, 2010) showed non-detectable values for the conventional oils, whereas oils from germinated seeds exhibited values of 5 ± 1 mg/kg HCN. These results showed the same trend as the areas of the identified cyanogenic glycosides using the untargeted LC-HRMS/MS approach in Table S1. Germination significantly increased the values of all cyanogenic glycosides found, namely amygdalin, linustatin, linamarin, and lotaustralin, but they were still below the threshold value, suggesting compliance with safety regulations. In contrast, Li et al., 2019 demonstrated that the germination process reduces the total content of cyanogenic glycosides, with significant decreases observed in linustatin, neolinustatin, and lotaustralin, but an increase in linamarin content. This indicates possible variations in the metabolic pathways of cyanogenic glycosides or environmental factors influencing the germination process and, consequently, suggests the need to assess oils from germinated seeds in terms of cyanogenic glycosides.

3.3. Antioxidants

3.3.1. High total polyphenol content and antioxidant capacities due to germination

Understanding the effects of germination on the antioxidant capacity and polyphenol content of oils is critical for harnessing their potential health benefits. Therefore, the antioxidant capacities were determined by ABTS, DPPH, and FRAP assays, and the total polyphenol content (TPC) was analyzed using Folin-Ciocalteu. Significant changes in antioxidant capacities and TPC were observed during the germination process. Table 1 shows that germinated linseed and sunflower seed oils exhibited remarkable increases in antioxidant activity compared to their conventional counterparts. Furthermore, TPC in germinated linseed and sunflower seed oils exhibited values of 572.8 ± 13.1 mg/kg and 139.5 ± 15.2 mg/kg, respectively, indicating their enhanced antioxidant potential, which suggests higher oxidative stability and shelf-life of the oils. These findings are consistent with recently published results reported by Zhang et al. (2023), who demonstrated similarly elevated TPC (236.30 ± 0.46 mg GAE/kg) and antioxidant activity (DPPH: 605.62 ± 0.39 μmol TE kg^{-1}, ABTS: 1864.41 ± 4.84 μmol TE kg^{-1}) in linseed oils after 5 days of germination. The linseed oils from germinated seeds tested in the present study had a higher TPC content, which may be due to the climatic conditions of the original seed. Overall, the results from these assays showed that linseed oil has higher antioxidant properties than sunflower seed oil.

Table 1
Total polyphenol content and antioxidant capacities of conventional and germinated linseed and sunflower seed oils. Unpaired t-tests, utilizing parametric tests with a FDR approach for multiple comparisons were conducted, and data are presented as mean ± SD. n = 3, *** = $p < 0.001$, **** = $p < 0.0001$.

Oil Sample	ABTS [μmol TE/kg]	DPPH [μmol TE/kg]	FRAP [mmol [Fe(II)/kg]	TPC [mg GAE/kg]	Recovery [%]
Conventional Linseed Oil	17.9 ± 1.1	9 ± 0.2	169.9 ± 12.9	15.4 ± 1.4	98.7
Germinated Linseed Oil	1612.1 ± 45.3****	609.9 ± 11.4****	3092.2 ± 85****	572.8 ± 13.1****	106.4
Conventional Sunflower Seed Oil	10.9 ± 1.5	10 ± 0.1	139.9 ± 17.2	12 ± 1.3	97.3
Germinated Sunflower Seed Oil	245 ± 31.7****	177.1 ± 8.1****	590 ± 42.9***	139.5 ± 15.2***	105.8

ABTS: 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay in μmol TE/kg, DPPH: 1,1-diphenyl-2-picrylhydrazyl assay in μmol TE/kg, FRAP: ferric reducing antioxidant potential assay in mmol Fe(II)/kg, TPC: total polyphenol content in mg gallic acid equivalents (GAE)/kg, Recovery of GAE in %.

3.3.2. Dynamic changes revealed by untargeted LC-HRMS/MS analysis

LC-HRMS was performed to detect phenolic substances within the oil samples, in order to adjust and optimize the polyphenol profile characterization into a quantitative MRM workflow (see 2.5.4). Another aim was to detect specific substances that were transferred to the oil due to germination.

Untargeted LC-HRMS/MS analysis revealed several compounds that were potentially synthesized during the germination process and subsequently incorporated into the pressed oils. Exploration of these compounds was facilitated by MZmine3.4.16, which enabled the precise identification and annotation of phenolic substances and phytochemicals. Table S1 outlines the presence of various substances, including oleuropein, umbelliferone, and plant sterols. While our primary focus was on pythochemicals, a germination-specific increase on the essential amino acid tryptophan was also detected. Significantly increased expression levels were observed after germination, indicating transformation and enrichment. Notably, plastochromanol-8 exhibited a substantial increase in the germinated linseed oil, highlighting its pronounced accumulation during germination. These findings underscore the dynamic biochemical changes that occur during seed germination and their profound impact on oil composition. Using an untargeted approach, a large number of phenolic substances were successfully identified. Further compounds were enriched in the germinated seed oils, confirming the complexity of the germination process. Secondary plant compounds serve as natural defense mechanisms against pathogens, pests, and herbivores, and attract pollinators (Miralpeix et al., 2013). Oleuropein has been shown by Coppa et al., 2020 to have antimicrobial, antioxidant, and anti-inflammatory properties and has been linked to the prevention of heart disease, improvement of lipid metabolism, and reduction of obesity. The observed increase in tryptophan may be attributed to protein degradation by proteases and synthesis of new enzymes during seed germination (Sibian, Saxena, & Riar, 2016). Plastochromanol-8 is mainly found in linseeds and has more pronounced antioxidant properties than α-tocopherol. They also play a crucial role in seedling development (Mène-Saffrané et al., 2010). To the best of our knowledge, this is the first study to detect significant changes in these compounds due to germination in oils, which may indicate potential health benefits and effects on oxidative stability. However, further

research is required to elucidate the nutritional and functional properties of these oils.

3.3.3. Specific polyphenol profiles detection by targeted LC-MS/MS

The comprehensive analysis conducted in this study represents an effort to elucidate the polyphenolic profiles of oils using LC-MS/MS multiple reaction monitoring (MRM) mode. Polyphenols are divided into specific categories including flavanols, flavones, flavonoids, lignans, phenolic acids, phenolic aldehydes, and stilbenoids. Comprehensive analysis revealed a substantial increase in the number of quantified polyphenols from germinated seed oils compared to conventional oils, with linseed oil showing an increase from 48 to 69 different polyphenolic species, and sunflower seed oil exhibiting an elevation from 47 to 66 polyphenols. This confirmed the incorporation

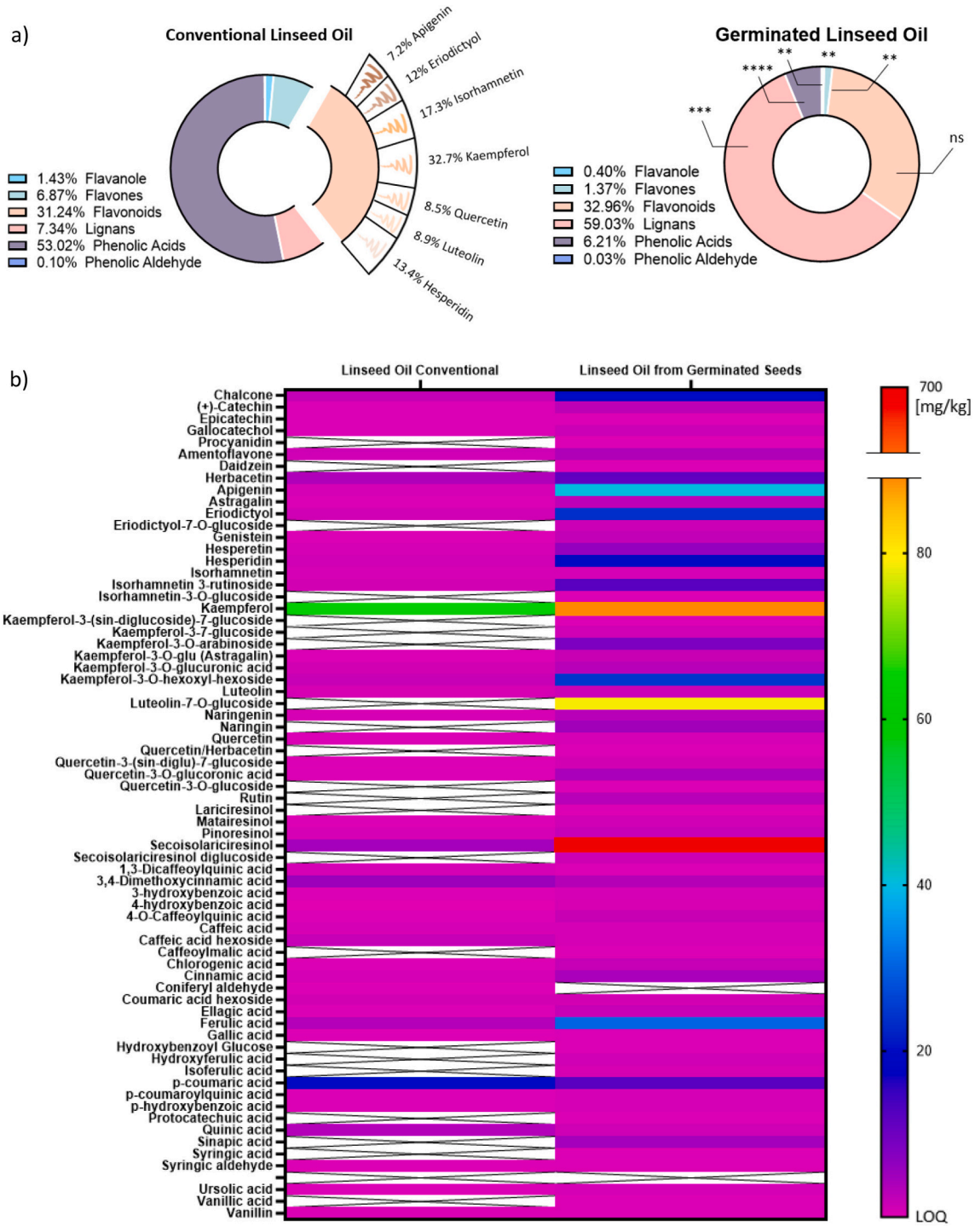


Fig. 2. Specific polyphenol profiles of conventional linseed oil and thus from germinated seeds. Pie charts in a) illustrate the mean distribution of polyphenol classes in conventional linseed oil and thus from germinated seeds. Unpaired *t*-tests, utilizing parametric tests with a FDR approach for multiple comparisons were conducted, with n = 3, ns = no significance, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. The heatmap in b) depicts the abundance of detected polyphenolic substances in mg polyphenolic substance/kg oil, which increases from purple to red and the crossed out white areas indicate <LOD and < LOQ. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of new polyphenolic substances into cold-pressed oils from germinated seeds, highlighting the dynamic nature of polyphenol production and composition in seeds. Overall, we observed distinct profiles for both linseed and sunflower seed oils with significant alterations following germination. The summed polyphenol content of conventional linseed oil exhibited 0.12 ± 0.01 mmol/kg, while germinated linseed oil showed 2.28 ± 0.34 mmol/kg. In comparison, sunflower seed oil contained 0.1 ± 0.01 mmol/kg, and germinated sunflower seed oil showed 0.95 ± 0.08 mmol/kg.

As depicted in Fig. 2a, the polyphenolic profile of conventional linseed oil was characterized by the predominant presence of phenolic acids (53.02%), followed by flavonoids (28.27%), and lignans (7.34%). Further analysis revealed specific polyphenolic compounds within the flavonoid category, including kaempferol (32.7%), isorhamnetin (17.3%), and hesperidin (13.4%). Linseed oil's most abundant phenolic substances were kaempferol (9.78 ± 1.73 mg/kg), followed by p-coumaric acid (19.17 ± 1.9 mg/kg). The most abundant specific substances aligned well with previously reported common polyphenolic substances

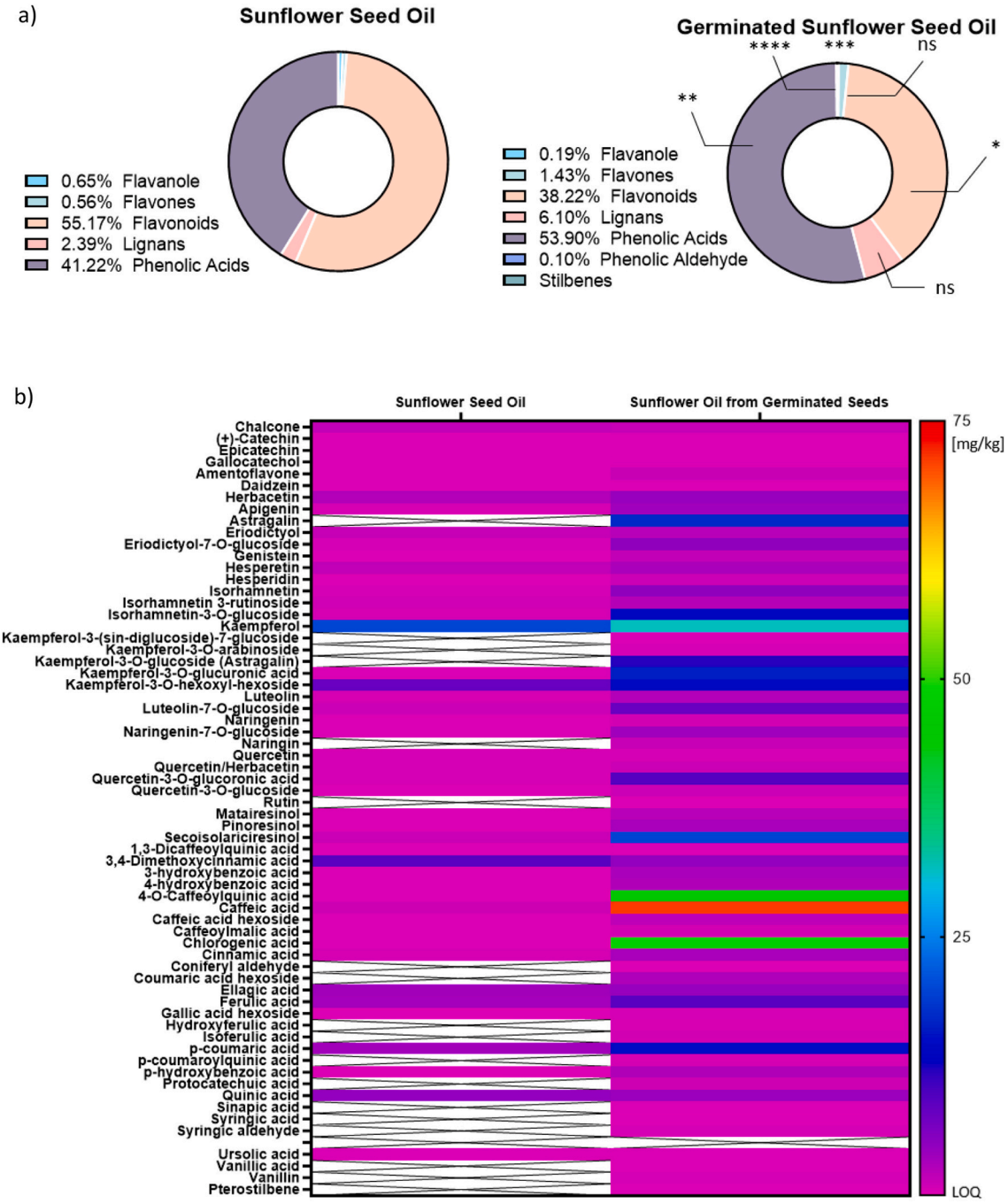


Fig. 3. Specific polyphenol profiles of conventional sunflower seed oil and thus from germinated seeds. The distribution of polyphenol classes in conventional oil and thus from germinated sunflower seeds is shown in pie charts in a), which show significant changes in the most pronounced phenolic classes. Unpaired t-tests, utilizing parametric tests with a FDR approach for multiple comparisons were performed, data are presented as mean values, n = 3, ns = no significance, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. The heatmaps in b) illustrate all quantified specific polyphenols in sunflower seed oil samples, revealing notable shifts in polyphenolic composition between conventional and germinated seed oils. The quantified values in mg polyphenolic substance/kg oil increase from purple to red, the crossed out white areas indicate <LOD and < LOQ. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in cold-pressed linseed oil (Hasiewicz-Derkacz et al., 2015). Upon germination, notable shifts occurred in the linseed oil profile, exhibiting significantly higher proportions of lignans (59.03%), followed by flavonoids (31.17%) and phenolic acids (6.21%). All individual polyphenols detected are shown in the heatmaps in Fig. 2b. In particular, linseed oil from germinated seeds displayed a remarkable elevation in secoisolariciresinol (650.53 ± 112 mg/kg, 142.3-fold change), kaempferol (102.3 ± 14.31 mg/kg, 10.46-fold change), and luteolin-7-O-glucoside (78.62 ± 4.5 mg/kg, not detected in conventional linseed oil), emphasizing the impact that germination has on the polyphenolic oil profile. Highly enhanced levels of secoisolariciresinol in linseed oil from germinated seeds have shown anti-inflammatory, anti-mutagenic, anti-microbial, anti-obesity, anti-hypolipidemic, and neuroprotective effects. Hano et al., 2017 showed that secoisolariciresinol is a very effective antioxidant, especially protecting against secondary oxidation products, making it a promising additive for edible oils. Consequently, the high concentration observed could influence the shelf-life of germinated linseed oil.

In contrast, as shown in Fig. 3a, conventional sunflower seed oil displayed a distinct polyphenolic composition, characterized by a high abundance of flavonoids (52.67%) and phenolic acids (41.22%). Germination further modified this profile, resulting in a significant increase in phenolic acid content (53.9%) and decrease in flavonoid content (37.85%). Notably, only marginal amounts of stilbenes were present in the germinated sunflower seed oil. The heatmap in Fig. 3b shows that conventional oil contained high levels of kaempferol (16.67 ± 1.9 mg/kg) and 3,4-dimethoxycinnamic acid (9 ± 1.49 mg/kg), while germinated sunflower seed oil exhibited a substantial rise in caffeic acid (71.41 ± 7.66 mg/kg, 72.23-fold increase), chlorogenic acid (49.34 ± 8.12 mg/kg), and 4-O-caffeoylquinic acid (44.38 ± 5.9 mg/kg). Phenolic acids, such as caffeic acid and chlorogenic acid, are associated with an enhancement of the oxidative stability of frying oils, as previously demonstrated by the addition of olive leave extracts with high amounts of phenolic acids (Chiou et al., 2007). This again indicates improved oxidative stability of polyphenol-rich oils from germinated seeds. The full list of all polyphenolic substances detected, including their concentrations, fold-changes, and significant changes, can be found in Table S2.

A comparison between linseed oil and sunflower seed oil revealed notable differences in the polyphenolic profiles. Linseed oil exhibited higher mean levels of p-coumaric acid (+15.51 mg/kg), while sunflower seed oil showed more pronounced concentrations of herbacetin (+6.89 mg/kg) and kaempferol-3-O-hexoxyl-hexoside (+6.25 mg/kg). Additionally, specific polyphenolic compounds were found exclusively in one oil or the other, such as astragalin and coniferyl aldehyde in linseed oil and daidzein, eriodictyol-7-O-glucoside, isorhamnetin-3-O-glucoside, and pterostilbene in sunflower seed oil. Alterations due to the germination process are illustrated in the heatmaps in Figs. 2 and 3. Specific polyphenolic substances were not present in the germinated sunflower seed oil, including procyanidin, kaempferol-3-7-glucoside, quercetin-3-(sinapoyl-diglucoside)-7-glucoside, and secoisolariciresinol diglucoside. Conversely, naringenin-7-O-glucoside, coniferyl aldehyde, and pterostilbene were absent in germinated linseed oil. These differences underscore the distinct effect that the type of seeds and their germination have on the polyphenolic composition of vegetable seed oils providing their first extensive quantitative characterization upon seed germination.

3.3.4. Detection of increase in tocopherols by HPLC/UV

The tocopherol profiles of the oils, depicted in Fig. 4, exhibited notable findings. Sunflower seed oil with 61.1 ± 4.9 mg/100 g oil contained significantly higher values than linseed oil,with 32.8 ± 1.6 mg/100 g. Consistent with the observations of Zhang et al., 2023, linseed oil displayed a predominance of γ-tocopherol, with discernible increases in α-tocopherol and δ-tocopherol levels after the germination process. In contrast, conventional linseed oil lacks δ-tocopherol entirely.

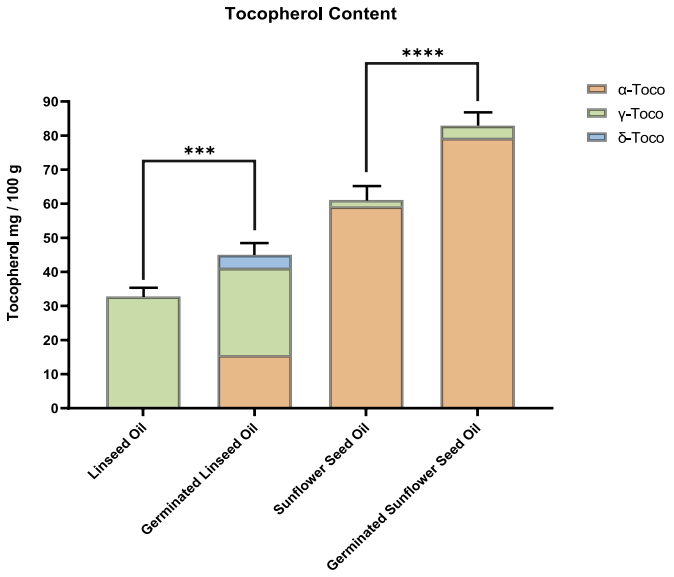


Fig. 4. Tocopherol profiles of oils by HPLC/UV with α, γ and δ-tocopherol detection. Unpaired t-tests, utilizing parametric tests with a FDR approach for multiple comparisons were conducted, and data are presented as mean ± SD in mg tocopherol/100 g oil, n = 3, *** = $p < 0.001$, **** = $p < 0.0001$.

Conversely, α-tocopherol is predominantly found in sunflower seed oil as it was shown previously by Aksoz, Korkut, Aksit, & Gokbulut, 2020. The results underscore significant elevations in tocopherol concentrations during germination, particularly γ-tocopherol, suggesting higher oxidative stability due to higher antioxidant content and, consequently, potential health benefits associated with the germination process (Thompson & Cooney, 2020).

3.3.5. Changes in chlorophyll and carotenoid content following germination

Chlorophyll and carotenoid content analyses, as depicted in Fig. S5, revealed significant alterations, particularly in the germinated sunflower seed oil. The mean chlorophyll content exhibited a substantial increase in germinated sunflower seed oil compared to its conventional counterpart from 0.01 to 21 ± 0.03 mg/kg. Linseed oil showed a pronounced increase from 0.61 to 16.4 ± 0.02 mg/kg after the germination process as well. This observation aligns with the findings of Herchi et al., 2015. Interestingly, although conventional linseed oil initially displayed higher chlorophyll levels than sunflower seed oil, this pattern was reversed following the germination process. Germination also significantly increased carotenoid content in both oil samples. However, germinated linseed oil demonstrated a higher abundance of carotenoids (15.7 ± 0.02 mg/kg) compared to sunflower seed oil, with a content of 14.1 ± 0.02 mg/kg, suggesting distinct phytonutrient transformations in induced by the germination process, dependent on the type of seed. Notably, the pronounced increase in chlorophyll and carotenoid content resulting from germination could also be visually distinguished by a color change from yellow to green.

3.4. Oxidative stability and shelf-life prediction

3.4.1. Peroxide and acid value analysis and free fatty acid determination

As expected, conventional linseed oil exhibited a higher peroxide value than sunflower seed oil, reflecting its lower shelf-life due to its higher susceptibility to oxidation. Significant decreases were observed in peroxide values for both linseed oil and sunflower seed oil after germination, as shown in Fig. S6. In linseed oil, peroxide values decreased from 17.5 ± 0.5 to 6 ± 0.4 mEq/kg, while in sunflower seed oil, decreased from 14 ± 0.7 to 8.4 ± 0.2 mEq/kg. Therefore, the germination process led to a considerable reduction in peroxide value, indicating improved oxidative stability.

For the acid value, a significant increase was noted in both germinated linseed oil and germinated sunflower seed oil compared to their conventional counterparts. In germinated linseed oil, the acid value increased from 3.7 to 37.2 ± 0.2 mg KOH/g, and in germinated sunflower seed oil, it increased from 3.3 to 15.1 ± 0.3 mg KOH/g. These findings suggest that the germination process has a notable impact on the acid value of the oils, potentially influencing their shelf-life and quality. Further investigations are required to establish the currently non-existent threshold limits for peroxide and acid values in oils from germinated seeds.

During germination, free fatty acid content in seeds can increase significantly, as observed in flax seeds by Wanasundara, Wanasundara, & Shahidi, 1999. The calculation of free fatty acids (FFA) from both the acid value and NMR results revealed consistent trends in our samples, as indicated by a high Pearson correlation coefficient (r) of 0.999 ($p < 0.05$). Linseed oil derived from germinated seeds exhibited higher FFA values than conventional linseed oil and sunflower seed oil, as shown in Fig. S7. Specifically, the mean FFA value derived from the acid value analysis was 1.85% for conventional linseed oil and notably increased to 18.44 ± 0.1% for germinated linseed oil. NMR analysis showed a similar trend, with mean FFA values of 1.12 ± 0.1% for conventional linseed oil and substantially higher values of 13.46 ± 0.6% for germinated linseed oil. Conversely, sunflower seed oils exhibited lower FFA values than linseed oils, with both conventional and germinated sunflower seed oils showing relatively lower FFA percentages using both analytical methods. These findings underscore the effect of seed germination on FFA content in oils, with germinated linseed oil showing the most pronounced increase in FFA levels. The increased presence of FFA in oils can affect the taste and odour or even lead to an off-taste when consumed (Mattes, 2011). It is important, therefore, to assess which limits are acceptable for consumers in terms of FFA. In addition, the impact of FFA on health is complex and varies depending on the type of fatty acid, but several studies showed that fatty acids play a significant role in human health and disease risk.

The findings from Shen et al., 2021 shed light on the preferential oxidation substrate in heated soybean oil, revealing that triacylglycerols (TGs) are preferentially oxidized over free fatty acids (FFA) despite the rapid hydrolysis and availability of FFA as substrates for oxidation. Since NMR results showed significant increases in FFA content in germinated linseed oil compared to conventional linseed oil, a lower susceptibility of germinated seed oils to oxidation might occur. The observed increase in FFA content could indicate enhanced hydrolysis during plant germination for energy production, leading to the release of FFAs from TGs. Collectively, these findings suggest that free fatty acid production is enhanced during seed germination.

3.4.2. *Impact of germination on lipid hydrolysis products analyzed by NMR*

A comprehensive evaluation of the acylglycerol structures in the studied oils was conducted through NMR analysis to assess the impact of germination on lipid hydrolysis, as expressed in Fig. 5a, by molar percentages of monoacylglycerol (MG), diacylglycerol (DG), TG, FFA, and glycerol (Gol). Significant decreases in TG content after germination were observed, with linseed oil dropping from 95.62 ± 0.17 to 77.49 ± 0.22% and sunflower seed oil from 96.88 ± 0.24 to 89.9 ± 0.06%. Conversely, a significant rise in 1-MG was observed in germinated linseed oil but not in sunflower oil. Furthermore, both oils exhibited significant increases in the 1,3-DG content. On the other hand, the signal associated with 1,2-DG structures showed a significant increase in germinated linseed oil, but not in sunflower oil. Overall, Fig. 5b illustrates a significant overall accumulation in DG content for germinated linseed oil from 1.11 ± 0.1 mg/g to 3.6 ± 0.62 mg/g and for sunflower seed oil from 0.42 ± 0.19 mg/g to 0.74 ± 0.09 mg/g. Ma, Sun, Wang, & Sun, 2022 demonstrated positive effects of high concentrated DG content in supplemented oils (20–40%), including reduced body weight and body mass index in healthy individuals, specific reductions in waist circumference and serum total cholesterol, and a significant correlation between the duration of DG-rich oil intake and weight loss effectiveness. In contrast, the analyzed oils from germinated seeds showed lower values than the ones reported by Ma et al., 2022, hence why their potential health effect needs to be investigated. In light of these findings, it is plausible that the germination process may lead to healthier oil supplementation due to the higher DG content.

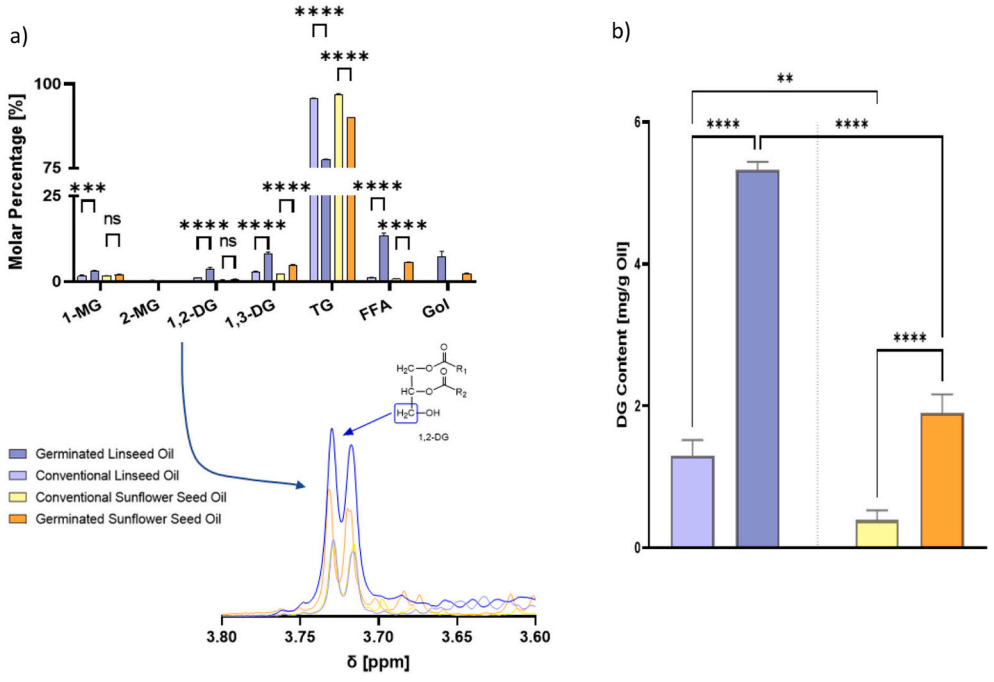


Fig. 5. NMR analysis of hydrolysis products in oils evaluated lipid composition changes, including monoglycerols (MG), diacylglycerols (DG), triacylglycerols (TG), free fatty acids (FFA), and glycerol (Gol) in molar percentage. The spectra shown illustrates 1,2-DG signal which showed the highest increase in linseed oil from germinated seeds. The observed overall increase in DG content is shown in b). One-way ANOVA was conducted, with Tukey post-hoc test, and data are presented as mean ± SD, n = 3, ns = no significance, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$.

3.4.3. Enhanced oxidative stability of germinated oils by Rancimat method

The oxidative stability and shelf-life of the oils were assessed using the Rancimat method, providing insights into the resistance of oil samples to oxidation under accelerated conditions. The measured oxidative induction time (OIT) values for conventional linseed oil align with the values previously reported by Varas Condori et al., 2020 at 110 °C, with an OIT of 2.18 ± 0.05 h and a calculated shelf-life of 90.6 days. Using this method, it was discovered that the oxidative stability of linseed oil from germinated seeds exhibited a notable increase compared to conventional linseed oil, with a 4.13 ± 0.25-fold increase in OIT, as shown in Fig. S8. Conversely, no significant increase was observed in sunflower seed oil, which can be attributed to its high stability in the conventional state. Symoniuk, Ratusz, & Krygier, 2017 found similar OIT values of 6.42 ± 0.06 h for conventional sunflower seed oil. Shelf-life predictions based on the Arrhenius equation revealed extended shelf-lives for germinated linseed oil (10.93 ± 0.8 months) and germinated sunflower seed oil (9.93 ± 0.53 months), compared to conventional linseed oil (2.66 ± 0.22 months) and sunflower seed oil (8.48 ± 0.37 months), respectively. The significant increase in oxidative stability observed in germinated linseed oil, as evidenced by the notable increase in OIT, underscores the potential of germination as a method to enhance the shelf-life of vegetable oils, highlighting the importance of considering this practice as a promising strategy to improve their oxidative stability. However, the high stability of conventional sunflower seed oil may limit the effects of germination on the oxidative stability of this particular oil, even if it had higher overall antioxidant levels, such as tocopherols, polyphenols, chlorophyll, and carotenoids.

3.4.4. GC–MS profiling of secondary lipid oxidation products

The secondary lipid oxidation product analysis conducted via GC–MS offered valuable insights into the oxidative degradation products present in the oils. The results revealed significant alterations in the composition of volatile oxidation compounds, indicative of ongoing oxidative processes within the oils, as depicted in Fig. 6.

Divergences were discernible in the profile of volatile secondary lipid oxidation products between linseed oil and sunflower seed oil as well as between their conventional and germinated variants. Notably, the hexanal and nonanal contents were markedly higher in linseed oil than in sunflower seed oil. Moreover, specific compounds, such as 1-octanol and 1-nonanol, were exclusively detectable in linseed oil. This may be related to their different fatty acid composition, as linseed oil is rich in linolenic acid, and these volatile compounds may originate from its oxidation.

The germination process included substantial changes in the content of secondary lipid oxidation products, indicating an overall increase in oxidative degradation induced by germination. This was shown by the enhanced levels of compounds, such as 2-butenal with an 11.3-fold increase in germinated linseed oil and 1-heptanol, with 1.5-fold higher values in germinated linseed oil and a 4-fold increase in germinated sunflower oil compared to conventional oils. However, certain compounds, such as heptanal, were solely detected in conventional oils and absent in germinated oils, suggesting the potential consumption or conversion of specific lipid oxidation products.

The overall higher content of secondary lipid oxidation products, especially in germinated sunflower oil, showed a different outcome from the Rancimat results. On the one hand, this could be due to the lack of correlation that Rancimat has been pointed out to have with certain oxidation parameters, such as secondary lipid oxidation products (Mancebo-Campos, Salvador, & Fregapane, 2007). Nevertheless, the predictive discrepancy between the two methods suggests that germinated sunflower seed oil has already undergone oxidative processes, leading to the formation of secondary oxidation products, as detected by GC–MS. However, the fatty acid profile could also explain the observed differences. Germinated sunflower seed oil showed an increase in the proportion of PUFAs, particularly linoleic acid, and a relative decrease in oleic acid content. This change in composition makes oil more susceptible to oxidation. Under accelerated oxidation conditions, as in the Rancimat method, all unsaturated fatty acids are likely to be affected by oxidation, leading to similar results between conventional and germinated sunflower seed oils. In addition, it was shown that the content of antioxidants, such as tocopherols, polyphenols, chlorophyll, and carotenoids, strongly increased because of germination. This influence could attenuate the effects of increased PUFA content on oxidation, so that the shelf-life is estimated to be similar despite the higher content of secondary lipid oxidation products. However, it should be noted that the effectiveness of antioxidants in oxidation may depend on several factors, including the type and concentration of antioxidants present, their interactions with other components of the oil, and specific oxidation pathways. Further research is needed to clarify the mechanisms underlying the role of antioxidants associated with the oxidative stability and shelf-life of oils from germinated seeds.

4. Conclusion

In conclusion, this study thoroughly investigated the profound effects of seed germination on the nutrient composition and oxidative stability of linseed and sunflower seed oils. Using advanced analytical techniques, we identified and quantified for the first time an extensive

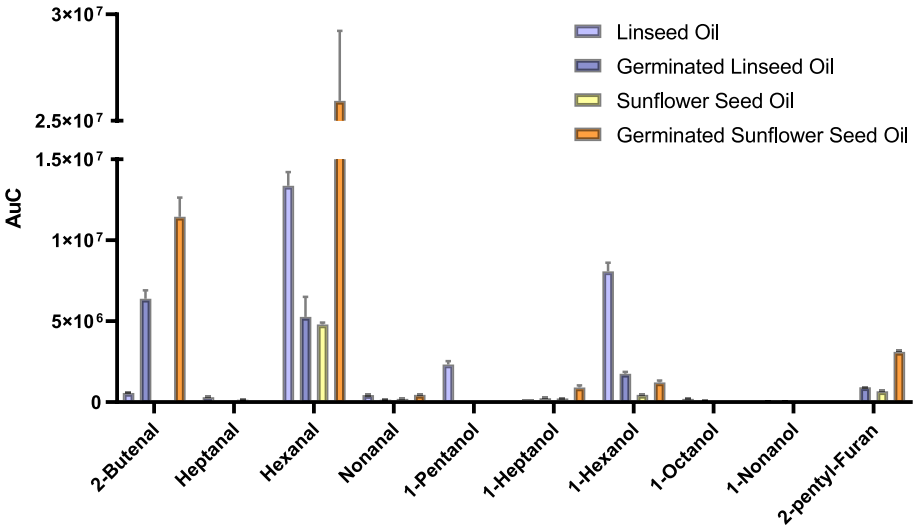


Fig. 6. Analysis of secondary lipid oxidation products by GC–MS analysis revealed drastic alterations in the studied oils. Data are presented as mean ± SD, $n = 3$.

set of polyphenol species in conventional linseed and sunflower seed oil as well as oils produced after the germination process. Germination led to a remarkable increase in antioxidant activity, total polyphenol content, tocopherol content, and free fatty acids, while at simultaneously causing significant changes in oxidative degradation products. Thus, we were able to show the potential effects of seed germination on the oxidative stability and shelf-life of linseed and sunflower oils.

Furthermore, our results emphasize the importance of seed germination as a processing step for improving the nutritional value. Overall, this study provides valuable insights into the chemical composition of oils from germinated seeds and paves the way for the development of novel approaches for enhancing the nutritional quality and shelf-life of oils for various applications in the food industry. Future research should focus on elucidating the mechanistic basis of these changes, investigating the effects of germination on other lipid components, enhancing the oil yield of germinated seeds and exploring strategies to optimize the germination process to maximize the health-promoting properties of oils.

CRediT authorship contribution statement

Tobias Pointner: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Katharina Rauh:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Arturo Auñon-Lopez:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Sanja Kostadinović Veličkovska:** Writing – review & editing, Methodology, Formal analysis. **Saša Mitrev:** Writing – review & editing. **Emilija Arsov:** Writing – review & editing. **Marc Pignitter:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research was funded in whole or in part by the Austrian Science Fund (FWF) [10.55776/P33636 and 10.55776/P34512]. For open access purposes, the author has applied a CC BY public copyright license to any author accepted manuscript version arising from this submission. This work was supported by Austria's Agency for Education and Internationalisation (OeAD, MK04/2022) via the bilateral Austrian-North Macedonian project with the title "Oxidomics-guided development of cold-pressed oils rich in antioxidants by improving manufacturing processes" (2022−2023). The authors would like to thank the NMR core facility of the Division of Pharmaceutical Chemistry at the University of Vienna for providing access to the NMR instruments and the Institute of Food Science at the BOKU - University of Natural Resources and Life Sciences for access to the Rancimat.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139790.

References

Aksoz, E., Korkut, O., Aksit, D., & Gokbulut, C. (2020). Vitamin E (α-, β + γ- and δ-tocopherol) levels in plant oils. *Flavour and Fragrance Journal, 35*, 504–510.
Aktar, T., & Adal, E. (2019). Determining the arrhenius kinetics of avocado oil: oxidative stability under Rancimat test conditions. *Foods, 8*, 236.
Alberdi-Cedeño, J., Ibargoitia, M. L., & Guillén, M. D. (2020). A global study by 1H NMR spectroscopy and SPME-GC/MS of the in vitro digestion of virgin flaxseed oil enriched or not with mono-, Di- or tri-phenolic derivatives. Antioxidant efficiency of these compounds. *Antioxidants, 9*, 312.
AOCS. (1995). *American Oil Chemists' Society, Official Methods And Recommended Practices*.
AOCS. (2017). AOCS official method Cd 3d-63: acid value of animal and vegetable fats and oils. In *Official Methods And Recommended Practices of the AOCS, Cd 3d-63*.
Belo, R. G., Tognetti, J., Benech-Arnold, R., & Izquierdo, N. G. (2014). Germination responses to temperature and water potential as affected by seed oil composition in sunflowers. *Industrial Crops and Products, 62*, 537–544.
Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry, 239*, 70–76.
Bewley, J. D., Bradford, K. J., Hilhorst, H. W., & Nonogaki, H. (2013). In *Seeds, 3* pp. 183–246). New York: Springer.
Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*, 25–30.
Cáceres, P. J., Peñas, E., Martinez-Villaluenga, C., Amigo, L., & Frias, J. (2017). Enhancement of biologically active compounds in germinated brown rice and the effect of sun-drying. *Journal of Cereal Science, 73*, 1–9.
Chiou, A., Salta, F. N., Kalogeropoulos, N., Mylona, A., Ntalla, I., & Andrikopoulos, N. K. (2007). Retention and distribution of polyphenols after pan-frying of French fries in oils enriched with olive leaf extract. *Journal of Food Science, 72*, S574–S584.
Commisson Regulation (EU) 2022/1364. (2022). Evaluation of the health risks related to the presence of cyanogenic glycosides in foods other than raw apricot kernels. *EFSA Journal. European Food Safety Authority, 17*, Article e05662.
Coppa, C., Gonçalves, B., Lee, S., Nunes, V., Gonçalves, C., Rodrigues, C., & Oliveira, C. (2020). Extraction of oleuropein from olive leaves and applicability in foods. *Quality Assurance & Safety of Crops and Food, 12*, 50–62.
Deutsche Gesellschaft für Fettwissenschaft (Ed.). (2020). *Fatty Acid Composition Of Important Vegetable And Animal Edible Fats And Oils*.
Dixit, S., Kendurkar, P. S., Vajpeyi, M., & Prasad, R. N. (2007). Variability in elite linseed (*Linum usitatissimum* L.) germplasm for oil content and fatty acid composition. *Indian Journal Of Agricultural Biochemistry, 20*, 35–37.
Dong, Y., Li, Y., Su, W., Sun, P., Yang, H., Li, Q., Du, S., & Yu, X. (2024). Differential metabolic networks in three energy substances of flaxseed (*Linum usitatissimum* L.) during germination. *Food Chemistry, 443*, Article 138463.
Eastmond, P. J., & Graham, I. A. (2001). Re-examining the role of the glyoxylate cycle in oilseeds. *Trends in Plant Science, 6*(2), 72–78.
Farag, M. A., Elimam, D. M., & Afifi, S. M. (2021). Outgoing and potential trends of the omega-3 rich linseed oil quality characteristics and rancidity management: a comprehensive review for maximizing its food and nutraceutical applications. *Trends in Food Science & Technology, 114*, 292–309.
Fruehwirth, S., Zehentner, S., Salim, M., Sterneder, S., Tiroch, J., Lieder, B., … Pignitter, M. (2020). In vitro digestion of grape seed oil inhibits phospholipid-regulating effects of oxidized lipids. *Biomolecules, 10*, 708.
Gliszczynska-Swiglo, A., Sikorska, E., Khmelinskii, I., & Sikorski, M. (2007). Tocopherol content in edible plant oils. *Polish Journal Of Food And Nutrition Sciences, 57*, 157–161.
Grüneis, V., Fruehwirth, S., Zehl, M., Ortner, J., Schamann, A., König, J., & Pignitter, M. (2019). Simultaneous analysis of epoxidized and hydroperoxidized triacylglycerols in canola oil and margarine by LC-MS. *Journal of Agricultural and Food Chemistry, 67*, 10174–10184.
Guo, S., Klinkesorn, U., Lorjaroenphon, Y., Ge, Y., & Na Yom, K. (2021). Effects of germinating temperature and time on metabolite profiles of sunflower (*Helianthus annuus* L.) seed. *Food Science & Nutrition, 9*(6), 2810–2822.
Hano, C., Corbin, C., Drouet, S., Quéro, A., Rombaut, N., Savoire, R., Molinié, R., Thomasset, B., Mesnard, F., & Lainé, E. (2017). The lignan (+)-secoisolariciresinol extracted from flax hulls is an effective protectant of linseed oil and its emulsion against oxidative damage. *European Journal of Lipid Science and Technology, 119*.
Hasiewicz-Derkacz, K., Kulma, A., Czuj, T., Prescha, A., Żuk, M., Grajzer, M., … Szopa, J. (2015). Natural phenolics greatly increase flax (*Linum usitatissimum*) oil stability. *BMC Biotechnology, 15*, 62.
Herchi, W., Arráez-Román, D., Trabelsi, H., Bouali, I., Boukhchina, S., Kallel, H., … Fernández-Gutierrez, A. (2014). Phenolic compounds in flaxseed: a review of their properties and analytical methods. An overview of the last decade. *Journal of Oleo Science, 63*, 7–14.
Herchi, W., Bahashwan, S., Sebei, K., Ben Saleh, H., Kallel, H., & Boukhchina, S. (2015). Effects of germination on chemical composition and antioxidant activity of flaxseed (*Linum usitatissimum* L) oil. *Grasas y Aceites, 66*, Article e057.
Holler, M., Alberdi-Cedeño, J., Auñon-Lopez, A., Pointner, T., Martínez-Yusta, A., König, J., & Pignitter, M. (2023). Polylactic acid as a promising sustainable plastic packaging for edible oils. *Food Packaging and Shelf Life, 36*, Article 101051.
Huang, Y., Mei, G., Zhu, K., Ruan, X., Wu, H., & Cao, D. (2024). Shading treatment during late stage of seed development promotes subsequent seed germination and seedlings establishment in sunflower. *Plant Science, 341*, Article 111996.
Joshi, R. (2018). Role of enzymes in seed germination. *International Journal of Creative Research Thoughts*, 2320–2882. IJC.

Kozłowska, M., Gruczyńska, E., Ścibisz, I., & Rudzińska, M. (2016). Fatty acids and sterols composition, and antioxidant activity of oils extracted from plant seeds. *Food Chemistry, 213*, 450–456.
Lall, R. K., Proctor, A., & Jain, V. P. (2009). A rapid, micro FAME preparation method for vegetable oil fatty acid analysis by gas chromatography. *Journal of the American Oil Chemists' Society, 86*, 309–314.
Leung, J., Fenton, T. W., & Clandinin, D. R. (1981). Phenolic components of sunflower flour. *Journal of Food Science, 46*, 1386–1388.
Li, X., Li, J., Dong, S., Li, Y., Wei, L., Zhao, C., Li, J., Liu, X., & Wang, Y. (2019). Effects of germination on tocopherol, secoisolarlciresinol diglucoside, cyanogenic glycosides and antioxidant activities in flaxseed (*Linum usitatissimum* L.). *International Journal of Food Science & Technology, 54*, 2346–2354.
Li, Z., Peng, S., Chen, X., Zhu, Y., Zou, L., Liu, W., & Liu, C. (2018). Pluronics modified liposomes for curcumin encapsulation: sustained release, stability and bioaccessibility. *Food research international (Ottawa, Ont.), 108*, 246–253.
Ma, W., Sun, J., Wang, J., & Sun, G. (2022). The effect of DAG oil replacing TAG oil on weight control and glycolipid metabolism: a meta-analysis of randomized controlled trials. *Journal of Functional Foods, 89*, Article 104931.
Mancebo-Campos, V., Salvador, M. D., & Fregapane, G. (2007). Comparative study of virgin olive oil behavior under Rancimat accelerated oxidation conditions and long-term room temperature storage. *Journal of Agricultural and Food Chemistry, 55*, 8231–8236.
María Landete, J., Hernández, T., Robredo, S., Dueñas, M., Las Rivas, B.d., Estrella, I., & Muñoz, R. (2015). Effect of soaking and fermentation on content of phenolic compounds of soybean (*Glycine max* cv. Merit) and mung beans (*Vigna radiata* L Wilczek). *International Journal of Food Sciences and Nutrition, 66*, 203–209.
Martínez-Yusta, A., & Guillén, M. D. (2014). A study by 1H nuclear magnetic resonance of the influence on the frying medium composition of some soybean oil-food combinations in deep-frying. *Food Research International, 55*, 347–355.
Mattes, R. (2011). Accumulating evidence supports a taste component for free fatty acids in humans. *Physiology & Behavior, 104*, 624–631.
Mène-Saffrané, L., Jones, A. D., & DellaPenna, D. (2010). Plastochromanol-8 and tocopherols are essential lipid-soluble antioxidants during seed desiccation and quiescence in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 17815–17820.
Miralpeix, B., Rischer, H., Häkkinen, S. T., Ritala, A., Seppänen-Laakso, T., Oksman-Caldentey, K.-M., … Christou, P. (2013). Metabolic engineering of plant secondary products: which way forward? *Current Pharmaceutical Design, 19*, 5622–5639.
Muhammad, N.*., Faqir Muhammad, A., Moazzam Rafiq, K., Muhammad, S., & Asad, R. (2011). *Antioxidant potential of bell pepper (Capsicum annum L.)-A review*.
Nieva-Echevarría, B., Encarnación, G., María J., M., & María D., G. (2017). *1H NMR and SPME-GC/MS study of hydrolysis, oxidation and other reactions occurring during in vitro digestion of non-oxidized and oxidized sunflower oil*.
ÖNORM EN 16160. (2010). Futtermittel — Bestimmung von Blausäure mittels HPLC. In *Institute, Austrian Standards*. Wien: Austrian Standards Institute.
Pająk, P., Socha, R., Gałkowska, D., Rożnowski, J., & Fortuna, T. (2014). Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chemistry, 143*, 300–306.
Parker, T. D., Adams, D. A., Zhou, K., Harris, M., & Yu, L. (2003). Fatty acid composition and oxidative stability of cold-pressed edible seed oils. *Journal of Food Science, 68*, 1240–1243.
Petraru, A., Ursachi, F., & Amariei, S. (2021). Nutritional characteristics assessment of sunflower seeds, oil and cake. Perspective of using sunflower oilcakes as a functional ingredient. *Plants, 10*, 2487.
Pignitter, M., Grosshagauer, S., & Somoza, V. (2019). Stability of vitamin E in foods. In P. Weber, M. Birringer, J. B. Blumberg, M. Eggersdorfer, & J. Frank (Eds.), *Vitamin E in Human Health* (pp. 215–232). Cham: Humana Press.
Pignitter, M., Stolze, K., Gartner, S., Dumhart, B., Stoll, C., Steiger, G., … Somoza, V. (2014). Cold fluorescent light as major inducer of lipid oxidation in soybean oil stored at household conditions for eight weeks. *Journal of Agricultural and Food Chemistry, 62*, 2297–2305.
Pino, L., Searle, B., Bollinger, J., Nunn, B., MacLean, B., & MacCoss, M. (2017). The Skyline ecosystem: Informatics for quantitative mass spectrometry proteomics. *Mass Spectrom Reviews, 39*(3), 229–244.
Prado, D. M. F. D., Almeida, A. B. D., Olivera Filho, J. G. D., Alves, C. C. F., Egea, M. B., & Lemes, A. C. (2020). Extraction of bioactive proteins from seeds (corn, sorghum, and sunflower) and sunflower byproduct: enzymatic hydrolysis and antioxidant properties. *Current Nutrition & Food Science*, 1–11.
Premnath, A., Narayana, M., Ramakrishnan, C., Kuppusamy, S., & Chockalingam, V. (2016). Mapping quantitative trait loci controlling oil content, oleic acid and linoleic acid content in sunflower (*Helianthus annuus* L.). *Molecular Breeding, 36*, 1–7.
Prescha, A., Grajzer, M., Dedyk, M., & Grajeta, H. (2014). The antioxidant activity and oxidative stability of cold-pressed oils. *Journal of the American Oil Chemists' Society, 91*, 1291–1301.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology & Medicine, 26*, 1231–1237.
Rico, D., Peñas, E., Del García, M. C., Martínez-Villaluenga, C., Rai, D. K., Birsan, R. I., … Martín-Diana, A. B. (2020). Sprouted barley flour as a nutritious and functional ingredient. *Foods, 9*, 296.
Sauer, D. B., & Burroughs, R. (1986). Desinfaction of seed surface with sodium hypochlorite. *Phytopatology, 76*, 745–749.
Schmid, R., Heuckeroth, S., Korf, A., Smirnov, A., Myers, O., Dyrlund, T. S., … Pluskal, T. (2023). Integrative analysis of multimodal mass spectrometry data in MZmine 3. *Nature Biotechnology, 41*, 447–449.
Sheeba, K., Saumya, C., Anamika, P., Mohd Kamran, K., & George, T. (2015). Sunflower oil: efficient oil source for human consumption. *Emergent Life Sciences Research, 1*(1), 1–3. Published on 30.
Shen, Q., Zhang, Z., Emami, S., Chen, J., Nobrega, L., de Moura Bell, J. M., & Taha, A. Y. (2021). Triacylglycerols are preferentially oxidized over free fatty acids in heated soybean oil. *npj Science of Food, 5*, 7.
Sibian, M. S., Saxena, D. C., & Riar, C. S. (2016). Effect of pre and post germination parameters on the chemical characteristics of Bengal gram (*Cicer arietinum*). *LWT - Food Science and Technology, 65*, 783–790.
Singh, K. K., Mridula, D., Rehal, J., & Barnwal, P. (2011). Flaxseed: a potential source of food, feed and fiber. *Critical Reviews in Food Science and Nutrition, 51*, 210–222.
Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). *[14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. 299* pp. 152–178).
Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.
Somsong, P., Santivarangkna, C., Tiyayon, P., Hsieh, C.-M., & Srichamnong, W. (2020). Assessing polyphenol components and antioxidant activity during fermented Assam tea ball processing. *Sustainability, 12*, 5853.
Symoniuk, E., Ratusz, K., & Krygier, K. (2017). Oxidative stability and the chemical composition of market cold-pressed linseed oil. *European Journal of Lipid Science and Technology, 119*.
Thompson, M. D., & Cooney, R. V. (2020). The potential physiological role of γ-tocopherol in human health: a qualitative review. *Nutrition and Cancer, 72*, 808–825.
Touré, A., & Xueming, X. (2010). Flaxseed Lignans: source, biosynthesis, metabolism, antioxidant activity, bio-active components, and health benefits. *Comprehensive Reviews in Food Science and Food Safety, 9*, 261–269.
Turgut Dunford, N. (2015). Oxidative stability of sunflower seed oil. In *Sunflower* (pp. 465–489). Elsevier.
Varas Condori, M. A., Pascual Chagman, G. J., Barriga-Sanchez, M., Villegas Vilchez, L. F., Ursetta, S., Guevara Pérez, A., & Hidalgo, A. (2020). Effect of tomato (*Solanum lycopersicum* L.) lycopene-rich extract on the kinetics of rancidity and shelf-life of linseed (*Linum usitatissimum* L.) oil. *Food Chemistry, 302*, Article 125327.
Wanasundara, P. K. J. P. D., Wanasundara, U. N., & Shahidi, F. (1999). Changes in flax (*Linum usitatissimum* L.) seed lipids during germination. *Journal of the American Oil Chemists' Society, 76*, 41–48.
Wang, H., Wang, J., Guo, X., Brennan, C. S., Li, T., Fu, X., … Liu, R. H. (2016). Effect of germination on lignan biosynthesis, and antioxidant and antiproliferative activities in flaxseed (*Linum usitatissimum* L.). *Food Chemistry, 205*, 170–177.
Wheeler, D. H. (1932). Peroxide formation as a measure of autoxidative deterioration. *Journal of the American Oil Chemists' Society, 9*, 89–97.
Yamamura, T., Arashima, M., Nakatani, K., Ishida, T., Yamada, F., & Nishiyama, T. (1995). 9 H -xanthene-2,7-diols as antioxidants for autoxidation of linoleic acid. *Journal of the American Oil Chemists' Society, 72*, 497–500.
Yang, J., Wen, C., Duan, Y., Deng, Q., Peng, D., Zhang, H., & Ma, H. (2021). The composition, extraction, analysis, bioactivities, bioavailability and applications in food system of flaxseed (*Linum usitatissimum* L.) oil: a review. *Trends in Food Science & Technology, 118*, 252–260.
Zhang, X., Zhang, Y., Sun, P., Su, W., Qu, Z., Dong, Y., … Yu, X. (2023). Effect of germination pretreatment on the physicochemical properties and lipid concomitants of flaxseed oil. *RSC Advances, 13*, 3306–3316.