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## **EIDESSTAATLICHE ERKLÄRUNG**

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TOBIAS POINTNER

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**ABSTRACT**

Cold-pressed vegetable oils are valued for their nutritional quality and bioactive minor compounds but are highly susceptible to oxidative deterioration due to their high polyunsaturated fatty acid (PUFA) content. This dissertation addresses strategies to enhance oxidative stability, from seed pretreatment and green extraction processes to packaging and predictive modeling of degradation.

In Part I, natural and process-level quality enhancement was investigated through germination and green extraction. Germination of linseed and sunflower seeds substantially increased antioxidant capacities and up to 37-fold more polyphenols, especially enhanced levels of secoisolariciresinol and a 4.1-fold longer predicted shelf-life. Furthermore, a novel approach to valorize press cake via green extraction produced cloudy linseed oil retaining suspended particles rich in antioxidants. These oils exhibited 6.3-fold higher polyphenol content, improved oxidative induction time (+52%) and lower hexanal levels (-93%), demonstrating that unfiltered, particle-enriched oils can combine sustainability and superior oxidative stability.

Part II explored packaging and additive-based stabilization. A 56-day storage study revealed that sunflower oil in polylactic acid (PLA) bottles showed comparable oxidative stability to polyethylene terephthalate (PET) and recycled PET, while conventional polyethylene (PE) and bio-PE led to faster oxidation. No harmful non-intentionally added substances were detected in PLA, supporting its use as a sustainable packaging alternative. Complementarily, fortification of PUFA-rich oils with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (PE(16:0/18:1)) increased oxidation induction time by up to 50%, reduced hydroperoxides and unsaturated aldehydes during storage and partially preserved tocopherols. Mechanistic experiments indicated that PE(16:0/18:1) decomposes hydroperoxides and traps carbonyls, exerting temperature-dependent antioxidative effects.

Part III focused on identifying robust biomarkers of oil deterioration from six cold-pressed oils over six months of storage under household conditions. Combining GC-FID, LC-MS/MS and GC-MS datasets with statistical approaches as LASSO, Random Forest and correlation analysis enabled the identification of degradation fingerprints. Fatty acid depletion, particularly of linoleic and  $\alpha$ -linolenic acid, emerged as the most consistent deterioration driver, while polyphenol degradation (e.g., p-coumaric acid, sinapic acid) and volatile formation (e.g., hexanal, 2,4-decadienal) revealed compound- and matrix-specific oxidation pathways. This

multi-marker approach was the first to systematically evaluate oil-type-specific biomarkers to further prediction of shelf-life.

In conclusion, the combined approaches of natural and sustainable process optimizations, material innovation and advanced data-driven biomarker identification, this thesis contributes to a deeper mechanistic understanding of lipid oxidation and provides practical strategies for the valorization of by-products and extension of the shelf-life of vegetable oils.

## ZUSAMMENFASSUNG

Kaltgepresste Pflanzenöle zeichnen sich durch ihre hohe ernährungsphysiologische Qualität und ihren Gehalt an bioaktiven Inhaltsstoffen aus, sind jedoch aufgrund ihres hohen Anteils an mehrfach ungesättigten Fettsäuren (MUFS) besonders anfällig für oxidative Zersetzung. Diese Dissertation befasst sich mit Strategien zur Verbesserung der Oxidationsstabilität – von der Saatvorbehandlung und grünen Extraktionsverfahren bis hin zu innovativen Verpackungslösungen und der Modellierung von Abbauprozessen.

Teil I untersuchte natürliche und prozessbezogene Ansätze zur Qualitätssteigerung durch Keimung und grüne Extraktion. Die Keimung von Leinsamen und Sonnenblumenkernen erhöhte die antioxidative Kapazität und den Polyphenolgehalt im Öl um das bis zu 37-fache, insbesondere durch eine Anreicherung von Secoisolariciresinol, was zu einer 4,1-fach längeren prognostizierten Haltbarkeit führte. Darüber hinaus ermöglichte ein neuartiger Ansatz zur Verwertung von Presskuchen durch grüne Extraktion die Herstellung von trübem Leinöl, das einen Teil suspendierte, antioxidantienreiche Partikel enthält. Diese Öle zeigten einen 6,3-fach höheren Polyphenolgehalt, eine um 52% verlängerte Oxidationsinduktionszeit und um 93% niedrigere Hexanalgehalte. Dies belegt, dass ungefilterte, partikelhaltige Öle einen nachhaltigen Ansatz mit verbesserter Oxidationsstabilität verbinden können.

Teil II widmete sich der Stabilisierung durch biobasierte Verpackungsmaterialien und funktionellen Zusätzen. Eine 56-tägige Lagerstudie zeigte, dass Sonnenblumenöl in Flaschen aus Polymilchsäure (PLA) eine vergleichbare Oxidationsstabilität wie in Polyethylenterephthalat (PET) und recyceltem PET aufwies, während konventionelles Polyethylen (PE) und bio-PE zu einer schnelleren Oxidation führten. In PLA wurden keine schädlichen nicht absichtlich eingebrachten Stoffe (NIAS) nachgewiesen, was den Einsatz als nachhaltige Verpackungsalternative unterstützt. Ergänzend führte die Anreicherung PUFA-reicher Öle mit 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamin (PE(16:0/18:1)) zu einer um bis zu 50% verlängerten Oxidationsinduktionszeit, einer Reduktion von Hydroperoxiden und ungesättigten Aldehyden während der Lagerung sowie zu einer teilweisen Erhaltung von Tocopherolen. Mechanistische Untersuchungen zeigten, dass PE(16:0/18:1) Hydroperoxide abbaut und Carbonylverbindungen abfängt und somit temperaturabhängige antioxidative Effekte entfaltet.

Teil III konzentrierte sich auf die Identifizierung robuster Biomarker. Sechs kaltgepresste Öle wurden dafür über sechs Monate unter haushaltsnahen Bedingungen gelagert. Durch die Kombination von GC-FID-, LC-MS/MS- und GC-MS-Datensätzen mit statistischen und maschinellen Lernverfahren (LASSO, Random Forest, Korrelationsanalyse) konnten ölspezifische Abbaumuster („Degradationsfingerabdrücke“) identifiziert werden. Der Abbau ungesättigter Fettsäuren, insbesondere von Linol- und  $\alpha$ -Linolensäure, erwies sich als konsistente Treiber der Oxidation, während der Abbau bestimmter Polyphenole (z. B. p-Cumarsäure, Sinapinsäure) und die Bildung flüchtiger Verbindungen (z. B. Hexanal, 2,4-Decadienal) substanz- und matrixspezifische Oxidationspfade aufzeigten. Dieser Multi-Marker-Ansatz stellt die erste systematische Studie dar, die zeigt, dass eine zuverlässige Vorhersage der Haltbarkeit nur durch die Integration mehrerer molekularer Indikatoren möglich ist.

Zusammengefasst leisten die vorgestellten Studien durch natürliche und nachhaltige Prozessoptimierung, Materialinnovationen und datengetriebene Biomarkeridentifizierung einen Beitrag zu einem tieferen mechanistischen Verständnis der Lipidoxidation sowie zu praktischen Strategien zur Valorisation von Nebenströmen und Verlängerung der Haltbarkeit kaltgepresster Öle.

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# 1 INTRODUCTION

Feeding a population expected to surpass nine billion will strain food systems unless production, processing and supply chains are re-engineered to reduce waste, adapt to climate change and improve resilience (Godfray et al. 2010; Maitin-Shepard 2020). At the same time our systems already drive substantial environmental pressures and it was estimated that roughly a third of global anthropogenic greenhouse-gas emissions is attributed to the food supply chain. This underscores the urgency of staying within planetary limits (Crippa et al. 2021; Li et al. 2024b). Meanwhile, an estimated 673 million people are facing chronic hunger, while 2.7 billion people are overweight. The demand for agricultural production is projected to rise by 50% by 2050 (World Health Statistics 2023) but humanity could require 80% more food by 2100 (Depenbusch & Klasen 2019). This paradox highlights the need for circular, resource-efficient food production and valorization of side-streams to minimize food loss along the whole supply chain. Globally, approximately 13–14% of food is lost between harvest and retail, while an additional 17% is wasted at the consumer level. In the EU alone, around 60 million tonnes of food are wasted every year—equivalent to about 131 kg per person and an economic cost exceeding €130 billion annually (Eurostat 2024). These highly alarming facts are summarised in Figure 1.

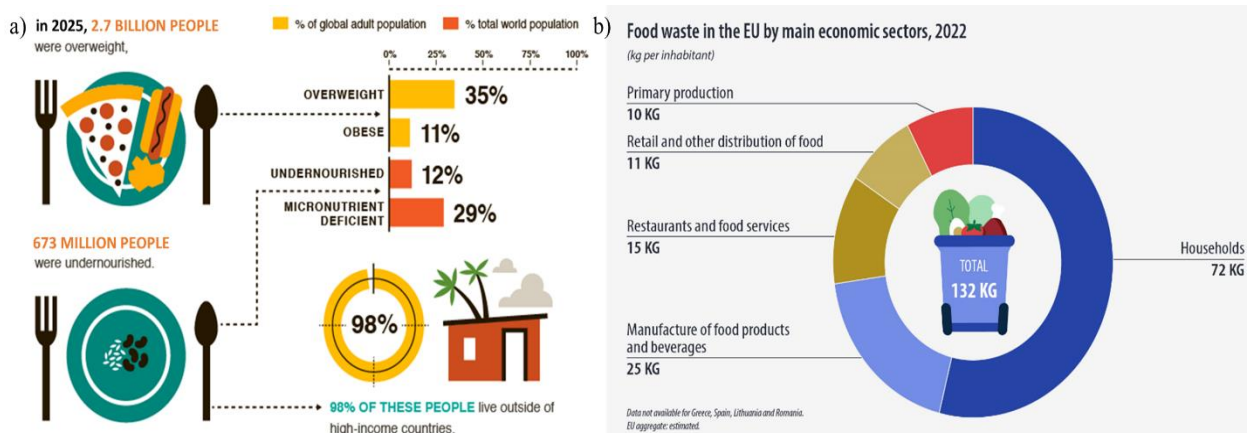


Fig. 1: a) global distribution of malnutrition and overnutrition (adapted from: FAO 2013; World Health Statistics 2023; Katharina Buchholz 2025). b) food waste in the European Union by main economic sectors in 2022 (Eurostat 2024).

These pressures coincide with shifting demographics and disease burdens (e.g., obesity, diabetes) that are changing consumer preferences toward healthier, more nutritious foods and higher trust in safety and authenticity (Whitfield et al. 2018; Poore & Nemecek 2018). Concurrently, the ‘fourth industrial revolution’ is transforming agri-food systems through big data, internet of things (IoT), sensors, artificial intelligence (AI), robotics and interoperable data flows. These technologies promise tighter traceability, fraud mitigation and faster, data-driven quality decisions across the global value chains (Schwab 2016; Hassoun et al. 2023; Serazetdinova et al. 2019). The sector is moving from single-parameter testing towards integrated analytics and digital infrastructures that convert data into decisions at speed and scale (Cook & Forzani 2018; Kamilaris et al. 2019).

Beyond production efficiency, sustainability also depends on material innovation and responsible packaging. In Europe, packaging represents nearly 40% of total plastic use, yet only about one-third is effectively recycled (OECD 2022). Plastic packaging for edible oils, often based on polyethylene terephthalate (PET), contributes significantly to fossil-based resource consumption and long-term environmental persistence, as PET can take centuries to degrade. Therefore, the exploration of biodegradable and renewable polymers derived from natural feedstocks such as corn, sugarcane or cassava starch. Among those, polylactic acid (PLA) has emerged as promising solution to fossil-based materials for food packaging (Taib et al. 2023). Still little is known about the interactions between food and biodegradable plastic alternatives and further research is needed regarding the migration of oligomers or microplastics into our daily food (Malafeev et al. 2023). Looking ahead, multiple impacts will be needed such as climate-smart production, circular resource use and side-stream valorization, all governed by science-based safety assessment and transparent regulation (Aschemann-Witzel et al. 2023; Onyeaka et al. 2023). Realizing these benefits will also require cross-disciplinary collaboration to evaluate new technologies (e.g. synthetic biology, additive manufacturing) and align them with nutrition, safety and costs (Habib et al. 2025).

Edible oil production exemplifies these challenges and opportunities of aligning nutritional quality with sustainability. The presscake residues are mostly treated as low-value by-products and used as animal feed or just going to waste. However, they contain valuable proteins, fibers and antioxidants such as polyphenols (Herchi et al. 2015; Fruehwirth et al. 2020). Their recovery and reintegration into food matrices support a circular bioeconomy and



contribute directly to the sustainable development goals (SDGs) on responsible consumption and production. Such valorization strategies can simultaneously enhance nutritional quality and oxidative stability while reducing environmental burden (Mannucci et al. 2023).

Given this focus on sustainability, resource efficiency and changing eating habits, cold-pressed vegetable oils have become more and more popular as people pay more attention to healthy lifestyles. They meet the growing consumer demand for minimally processed, „clean-label“ fats, yet pose practical challenges related to yield, oxidative stability and standardization across diverse oilseeds and pressing conditions (Savoire et al. 2013). According to the Codex Alimentarius (Codex-Stan 210-1999), cold-pressed oils must be produced solely by mechanical procedures, typically via screw-pressing, with minimal heat input and without the use of organic solvents. Processing parameters, such as moisture, temperature and screw speed strongly influence oil yield, sediment formation and early oxidation indices as peroxide value (PV) and acid value (AV) or free fatty acid (FFA) content, which makes quality control from press to bottle complicated (Wen et al. 2023; Rabadán et al. 2018; Kabutey et al. 2024). However, this process preserves a larger proportion of minor constituents compared to refined oils, resulting in oils naturally enriched in bioactive compounds such as polyphenols, tocopherols, carotenoids and chlorophylls (Rombaut et al. 2015). These compounds contribute to antioxidant potential and health-promoting properties (Pérez-Gálvez et al. 2020; Rodilla et al. 2023). Cold-pressed oils are usually high in polyunsaturated fatty acids (PUFAs) and its supplementation has been linked to antioxidant, anti-inflammatory and cardiometabolic benefits (Rabiej-Kozioł et al. 2023; Vukic & Vujasinovic 2023). However, the substances that make these oils nutritionally valuable also render them particularly susceptible to oxidative degradation. The abundance of high contents of linoleic and  $\alpha$ -linolenic acid (ALA) accelerates autooxidation and photooxidation, which shorten shelf-life under typical storage conditions (Pignitter et al. 2019). Moreover, some minor compounds such as chlorophylls can shift from antioxidant to prooxidant roles under light (Grajzer et al. 2020).

Edible oils have become an essential part of our cuisine, but their susceptibility to oxidation poses a constant challenge for manufacturers and consumers. Achieving good nutritional value requires a balance between minimal processing, long shelf-life and sustainable use of resources. Despite advances in analytical chemistry and food technology, routine quality

control still relies heavily on individual markers (e.g. hexanal) and regular testings, which cannot detect early degradation of antioxidants or oil-specific degradation pathways due to the still lacking understanding in lipid oxidation pathways. Furthermore, strategies as seed germination, by-product utilisation and the use of biodegradable or functional packaging materials remain little studied yet.

This dissertation addresses these gaps by developing an integrated approach that combines natural processing optimizations (germination and green extraction from oil press cake), material-based stabilization (aminophospholipid fortification and PLA packaging) and big data dependent biomarker modeling. Together, these strategies aim to enhance nutritional quality, oxidative stability and the predictability of shelf-life in edible oils, contributing to a more sustainable and scientifically grounded production.

## **1.1 LIPID OXIDATION**

In edible oils, quality loss is dominated by lipid oxidation, which proceeds via the two principal routes autoxidation (free-radical chain) and photooxidation (sensitized  $^1\text{O}_2$  chemistry). Because cold-pressed oils are PUFA-rich, the autoxidation pathway is especially consequential for shelf-life and sensory change (Frankel 1980; Choe & Min 2006).

Autoxidation follows the classical initiation–propagation–termination scheme as illustrated in Figure 2. An initiator abstracts a hydrogen from a bis-allylic site to form  $\text{L}^\bullet$ , which adds  $\text{O}_2$  to give  $\text{LOO}^\bullet$ . This peroxy radical then abstracts  $\text{H}^\bullet$  from another lipid, yielding a hydroperoxide ( $\text{LOOH}$ ) plus a new  $\text{L}^\bullet$ , sustaining the chain. Radical–radical coupling ( $\text{L}^\bullet + \text{L}^\bullet$ ;  $\text{LOO}^\bullet + \text{LOO}^\bullet$ ) terminates the process. Over time,  $\text{LOOH}$  accumulates and then declines as it decomposes (metal/heat-catalyzed) to secondary products as aldehydes, ketones, alcohols or acids, that drive rancid odor or flavor (Chaiyasit et al. 2007). In practice, lipid autoxidation is a network of interlocking reactions that fits the classical growth/branching/termination picture (Farmer et al. 1942). The rate of peroxidation is governed not only by fatty-acid composition ( $\text{PUFA} \gg \text{MUFA}$ ) but also by the balance of pro- and antioxidants, oxygen partial pressure, interfacial area (oil/air) and storage conditions (temperature, light, water activity) (Belitz et al. 2008; Choe und Min 2006; Pignitter et al. 2014). During initiation, hydrogen abstraction at bis-allylic sites yields  $\text{L}^\bullet$ , in PUFA, radical delocalization promotes double-bond migration and formation of conjugated dienes, which rise with  $\text{LOOH}$  during propagation. As time proceeds,

LOOH undergoes metal/heat-catalyzed decomposition to secondary and even tertiary products. LOOH themselves are odorless, but the resulting volatile aldehydes and ketones are highly odor-active at low concentrations, which support sensory decline (Belitz et al. 2008; Frankel 2005). During the induction period, endogenous and exogenous antioxidants (e.g., tocopherols, phenolics) quench peroxy radicals. As they deplete, propagation accelerates, seen as a steep rise in LOOH and  $O_2$  consumption. With continued reaction, termination and LOOH decomposition dominate. Primary markers plateau or decline while secondary volatiles and acid value increase, and PUFAs steadily decrease (Frankel 2005); Pignitter & Somoza 2012). These kinetics are summarized in the classical autoxidation process over time in Figure 3.

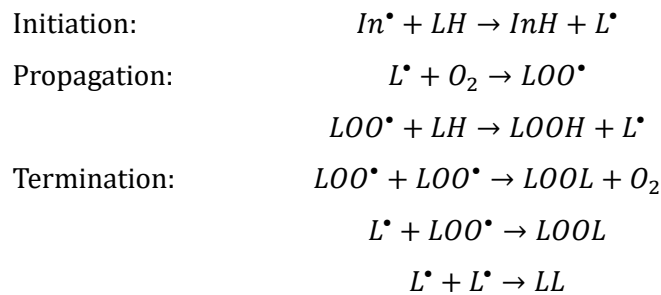


Fig. 2: Simplified scheme of the radical chain reaction (Chaiyasit et al. 2007).

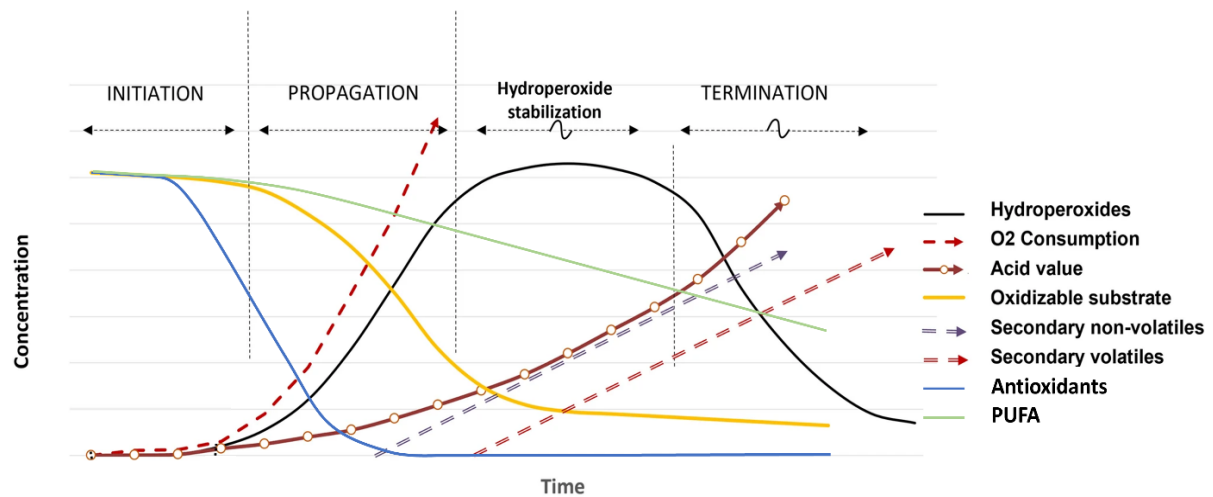


Fig. 3: Classical autoxidation pathway over time (adapted from: Musakhanian et al. 2022; Pignitter & Somoza 2012)

In addition to autoxidation, photo-oxidation can occur in the presence of light and photosensitizers such as chlorophylls. When exposed to UV or visible light, these sensitizers (Sen) absorb energy and reach an excited singlet state ( $\text{Sen}^*$ ), as it is shown in Figure 4 (Wang et al. 2023). The excited molecule can then initiate oxidation via two competing pathways.

In the type I mechanism, the excited sensitizer directly reacts with a substrate by abstracting a hydrogen atom or an electron, producing free radicals that trigger the autoxidation chain. In the type II mechanism, its energy is transferred to ground-state triplet oxygen ( $^3\text{O}_2$ ), generating singlet oxygen ( $^1\text{O}_2$ ), which is a highly reactive form that can oxidize lipids up to 1500 times faster than triplet oxygen (Choe und Min 2006). The balance between type I and type II photo-oxidation depends on the Sen structure, substrate composition and oxygen concentration (Frankel 1980).

$^1\text{O}_2$  reacts directly with PUFAs through an ene-reaction, forming allylic LOOHs, as illustrated in Figure 5 (Frankel, 1998). This reaction leads to double-bond rearrangement and unlike autoxidation, can also generate unconjugated double bonds. In accordance with this mechanism, twice as many LOOH can form per unsaturated fatty acid as there are isolated double bonds in the molecule (Akoh und Min 2002).

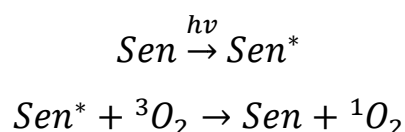


Fig. 4: Photooxidation type-2: the triplet oxygen ( $^3\text{O}_2$ ) gets raised in its excited singlet state through the reaction with a light-activated sensitizer (Foote 1968).

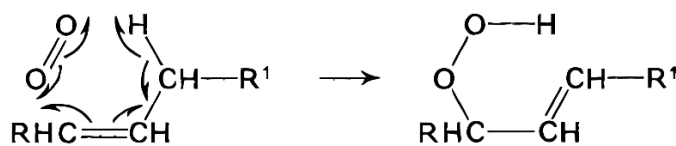


Fig. 5: Reaction of singlet oxygen directly with an unsaturated fatty acid (Belitz et al. 2008).

Apart from autoxidation and photo-oxidation, enzymatic oxidation may also occur when lipoxygenase (LOX) and hydroperoxidase enzymes are present. LOX catalyzes hydrogen abstraction from bis-allylic methylene groups in PUFA via reduction of its Fe(III)–OH cofactor, forming peroxy radicals that are subsequently reduced to hydroperoxides (Lampi et al. 2020).

## 1.2 ANTIOXIDANTS

Antioxidants are compounds that slow or prevent oxidation by scavenging reactive species or interrupting radical chains. Physiologically seen, they modulate reactive oxygen species (ROS) and oxidative stress (Rhee 1999). While excessive ROS damage lipids, proteins and DNA, moderate ROS act as signaling molecules, activating ERK/MAPK and redox-sensitive transcription factors (e.g. NF- $\kappa$ B), coordinating adaptive defenses (Loukili et al. 2010). In lipid matrices, antioxidants work mainly by H $\cdot$ /e $^{-}$  donation to peroxy radicals (chain-breaking), metal chelation (limiting LOOH decomposition) and  $^1\text{O}_2$  quenching in illuminated systems. Their effectiveness depends on antioxidant type and concentration as well as environmental factors (O $_2$ , light, temperature, interfacial area) (Choe und Min 2006).

In foods, processing and storage conditions have a high impact on the antioxidant content depending on the technology. Effects range from drastic losses due to heat-sensitivities or increases in carotenoid availability or phenolic-linked activity due to milder processings or even health related consequences (Poljsak et al. 2021). Especially in cold-pressed oils it is important to retain endogenous antioxidants (tocopherols, polyphenols, carotenoids, chlorophylls), which aid stability. However, light exposure can turn pigments into photosensitizers, so packaging, oxygen control and temperature remain crucial (Pignitter & Somoza 2012).

Tocopherols are the primary chain-breaking antioxidants in many seed oils. They react rapidly with LOO $\cdot$  to form relatively unreactive tocopheroxyl radicals and delay propagation. Profiles ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) vary by oil type and are sensitive to processing and storage (Choe und Min 2006). Carotenoids, such as  $\beta$ -Carotene, lutein, zeaxanthin and lycopene quench  $^1\text{O}_2$  and contribute to the specific oil type colors. Levels differ widely among oils and decline with heat and light exposure. Protective effects are context-dependent and carotenoids can interact with other pigments or phenolic substances (Herchi et al. 2015). Chlorophylls are lipophilic

pigments, which are responsible for green colors. In the dark they contribute together with phenolics to antioxidant effects, but under light + O<sub>2</sub> they can act as photosensitizers and promote type-II photooxidation. Pheophytin, commonly abundant in cold-pressed oils, shows similar behavior and forms from chlorophyll during heating or longer storage under light (Vukic und Vujasinovic 2023).

Polyphenols are often grouped as phenolic acids, flavonoids, stilbenes and lignans. Phenolic acids can be further subdivided into hydroxybenzoic and hydroxycinnamic acids and flavonoids into flavanols, flavanones, flavones, flavonols, isoflavones and chalcones as shown in Figure 6. Several hundred polyphenols are known nowadays and many of them occur as glycosides or esters. Their antioxidant activity depends on number and position of –OH groups and pH (Belitz et al. 2008). Diets rich in phenolics are associated with health-promoting effects (e.g., cardiometabolic, anti-inflammatory) and phenolic profiles vary with botanical origin, cultivar and geography (Bettaieb et al., 2023). In vegetable oils, polyphenols influence oxidative level, interaction with free fatty acids and sensory properties, making their quantification and profiling increasingly important for quality research (Rezig et al. 2022).

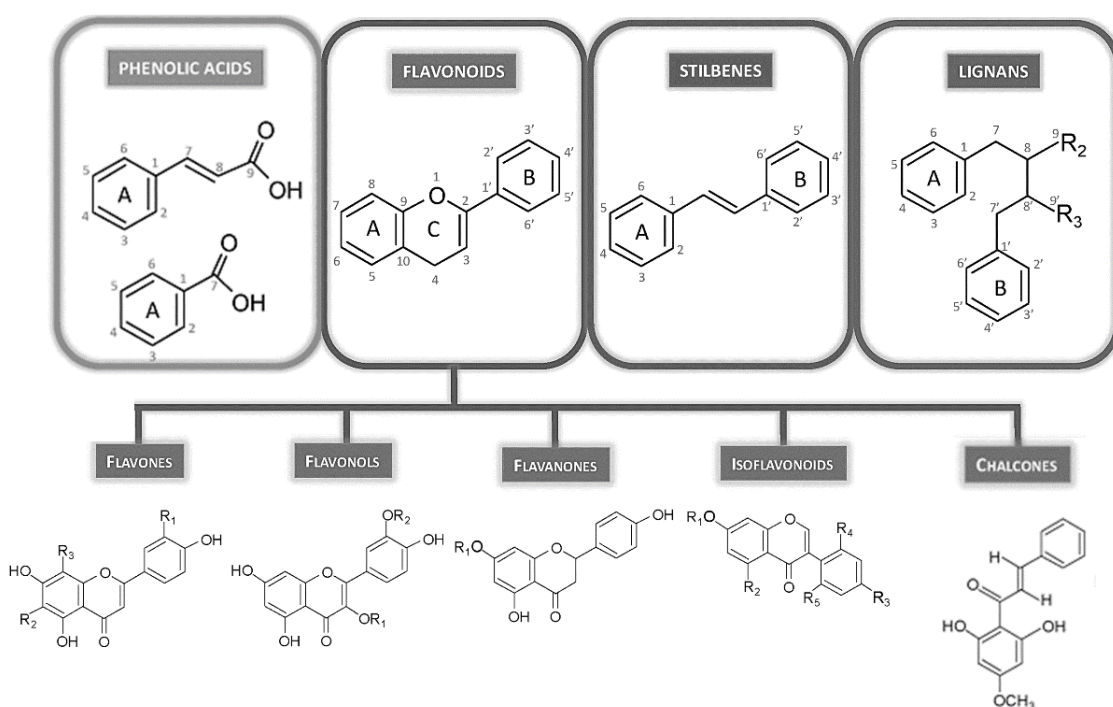


Fig. 6: Classification of polyphenols and sub-groups of flavonoids (adapted from: Di Lorenzo et al. 2021)

### 1.3 ANALYTICAL TOOLS FOR SHELF-LIFE PREDICTION OF COLD-PRESSED OILS

Shelf-life represents the period during which a food maintains acceptable quality being within legal microbiological regulations and nutritional and sensory limits under defined storage and distribution conditions (Man 2015). Its end point is typically identified when one or more critical attributes cross a rejection limit, whether due to sensory rancidity, nutrient loss or safety concerns. In edible oils, shelf-life is governed primarily by lipid oxidation and, to a lesser extent, hydrolytic and polymerization reactions (Frankel 2005). Temperature, oxygen availability, light exposure, moisture, and packaging permeability are dominant extrinsic factors, while fatty-acid unsaturation, minor antioxidant content (tocopherols, polyphenols, carotenoids), and processing history act as intrinsic determinants (Choe und Min 2006).

Refined oils generally exhibit longer shelf-lives with often exceeding 12 months, because the refining process removes pigments, metals, and pro-oxidants, though it also depletes natural antioxidants. Cold-pressed oils, rich in PUFAs and bioactives, show greater nutritional value but markedly lower oxidative stability; realistic commercial shelf-lives range from 3 to 9 months under ambient storage, depending on packaging and light exposure (Velasco und Dobarganes 2002; Pignitter et al. 2014). For lipid-driven deterioration, accelerated shelf-life testing commonly increases temperature and describes kinetics with the Arrhenius equation, which is shown in Equation 1 (Aktar und Adal 2019). However, this approach assumes single rate-limiting temperature dependence and constant reaction mechanisms. In practice, lipid oxidation pathways shift with light exposure, oxygen availability and packaging properties. These factors cannot be represented solely by accelerated temperature testings.

$$\text{Equ. 1: } k = Ae^{\frac{-E_a}{RT}}$$

where

- $k$  is the rate constant
- $T$  is the absolute temperature
- $A$  is the pre-exponential factor or Arrhenius factor
- $E_a$  is the molar activation energy
- $R$  is the universal gas constant

Conventional shelf-life evaluation in edible oils still relies on a limited set of chemical indices and periodic sampling. While these parameters are valuable for batch comparison and specification control, they remain insufficient for real-time or predictive assessment of oxidative stability (Man 2015). Traditionally, univariate markers such as the peroxide value (PV), anisidine value, acid value (AV), conjugated dienes and oxidative induction times (OIT) obtained from Rancimat or other accelerated protocols have been used to extrapolate room-temperature stability. Although these tools are simple and standardized, they capture only a narrow view of the complex, multidimensional chemistry of lipid oxidation and antioxidant degradation. Therefore, in recent years, the assessment of oil quality and stability has shifted from classical univariate indices towards high-information analytical platforms that provide multidimensional insight into oxidative processes. Spectroscopic, chromatographic and sensor-based techniques including Fourier transformation infrared spectra (FT-IR, FT-NIR), fluorescence spectroscopy, nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC)-MS systems, which could generate complementary chemical fingerprints (Lim et al. 2025; Esteki et al. 2020; Gonzalez Viejo & Fuentes 2022). These datasets enable chemometric modeling of degradation kinetics and sensory changes, yet the selection of reliable chemical markers remains challenging. Correlations between analytical indices and perceived rancidity are highly matrix-dependent, and no universal marker has been established (Kharbach et al. 2023). Consequently, multivariate and machine-learning (ML) approaches are increasingly applied to integrate such big data to identify robust markers to better understand lipid oxidation (Zhang et al. 2025).

In complex food systems such as cold-pressed oils, quality loss is driven by numerous interacting chemical reactions that generate a multitude of measurable compounds. Many of these substances change in a quantifiable manner with the duration of storage or sensory deterioration (Li et al. 2024). Such compounds, whether originating from lipid oxidation, antioxidant depletion or any secondary transformations could serve as biomarkers of degradation. In a predictive context, biomarkers act as informative proxies for overall stability or sensory food quality, enabling accelerated or non-destructive estimation parameters. The identification of reliable biomarkers requires statistical discrimination between features that merely fluctuate and those that genuinely track deterioration. Early approaches relied on correlation or univariate regression between individual analytes and storage time or rancidity scores. Nowadays however, multivariate or ML methods are capable of handling large variable



spaces, collinearity and non-linear relationships (Lin et al. 2023). These data-driven approaches could integrate chemical fingerprints from multiple analytical platforms and identify compounds whose concentration patterns most effectively describe the progression of oxidation or antioxidant loss.

While classical multivariate calibration techniques such as principal component analysis (PCA) or partial least squares (PLS) have long been used to reduce dimensionality and visualize trends, recent advances in ML enable flexible, non-linear modeling of complex pathways. ML approaches learn relationships between predictor variables such as spectral intensities or compound concentrations and target variables such as storage time, quality class or sensory acceptance. In this way, they provide both classification and quantitative prediction capabilities that extend beyond traditional regression models. Among available algorithms, feature-selection and ensemble methods have proven particularly effective for food-quality modeling (Zhang et al. 2025; Li et al. 2024).

Random forest regression (RFR) is a non-parametric ensemble learning method that constructs numerous decision trees on bootstrapped subsets of data and predictor variables (Breiman 2001). Each tree contributes a partial prediction, and the aggregated output provides a robust estimate less sensitive to noise or outliers, which is shown in Figure 7. RFR inherently models non-linear interactions and generates an importance score for each variable, quantifying its contribution to model accuracy or node purity. These scores enable direct ranking of potential biomarkers even when complex dependencies exist among predictors (Zhou et al. 2023).

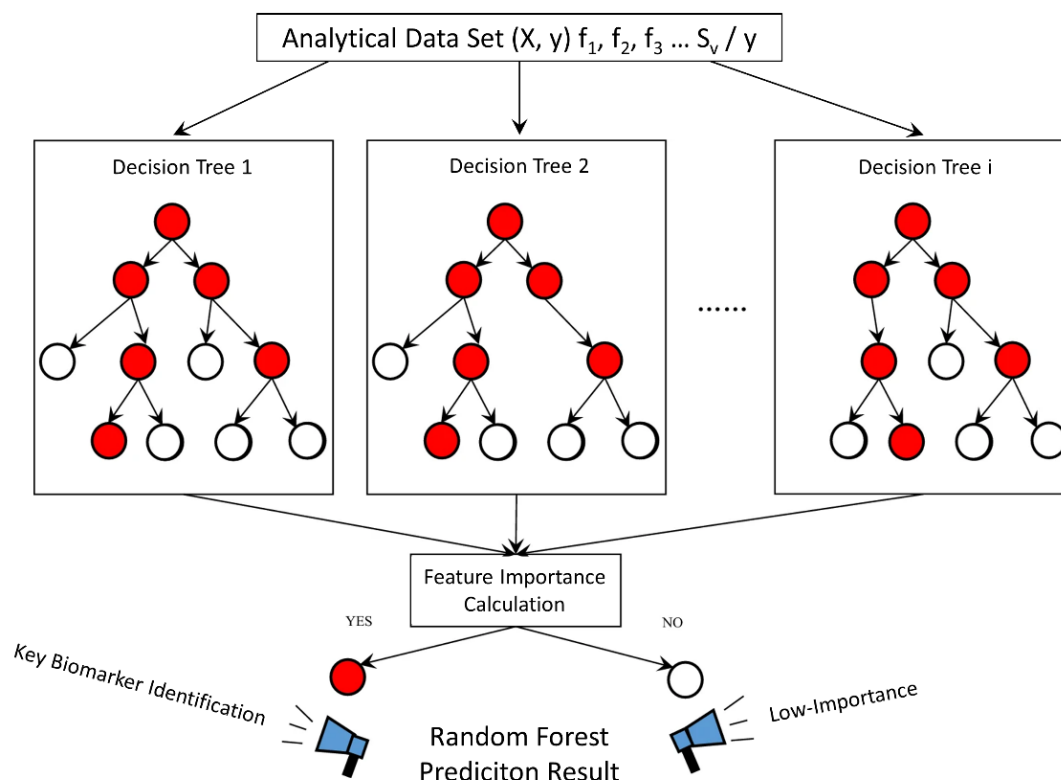


Fig. 7: Schematic overview of Random Forest regression (RFR) modeling for biomarker identification, with the ensemble of decision trees, feature importance calculation and selection of key markers (adopted from Zhou et al. 2023)

In contrast to the non-linear and ensemble-based nature of RFR, regression-based feature-selection methods such as the Least Absolute Shrinkage and Selection Operator (LASSO) identify predictive variables within a linear modeling framework. LASSO regression performs linear modeling with an  $L_1$ -norm penalty that constrains the sum of absolute coefficient values (Ranstam & Cook 2018). This regularization shrinks less informative coefficients toward zero, thereby performing embedded variable selection while minimizing overfitting in high-dimensional data. The corresponding LASSO objective function is shown in Equation 2. Although originally a statistical regularization approach, LASSO is widely applied in chemometrics and ML workflows for variable selection and model simplification in high-dimensional datasets. LASSO is particularly suited to datasets where the number of features exceeds the number of samples, which is a typical scenario in omics-type profiling (Li et al. 2024).

$$\text{Equ. 2: } \min_{\beta \in \mathbb{R}^p} \left\{ \frac{1}{N} \|y - X\beta\|_2^2 + \lambda \|\beta\|_1 \right\}$$

where

- $y$  = vector of observed responses (e.g., storage time or oxidation index)
- $X$  = matrix of predictors (e.g., compound intensities)
- $\beta$  = coefficient vector
- $N$  = number of samples (normalization term)
- $\lambda$  = regularization parameter controlling sparsity (penalty strength)

Together, these tools provide complementary perspectives on new biomarker discovery. Despite these methodological advances, practical challenges remain in translating data-driven modeling into industrial food-quality control. Routine systems still rely on offline, single-parameter assays such as PV and accelerated oxidation tests that emphasize temperature while neglecting the combined effects of light exposure, oxygen intake and packaging interactions. Moreover, ML approaches are often applied only in exploratory research and rarely validated across diverse oil matrices or integrated with multi-sensor analytical data.

## 1.4 USEFUL METHODS

To characterize the nutritional quality and oxidative stability of cold-pressed oils, a combination of complementary analytical techniques is required. This chapter describes the most relevant methods applied in this field, covering the determination of fatty acid composition, antioxidant content and secondary lipid oxidation products, as well as supporting assays and instrumental approaches used to evaluate degradation kinetics and shelf-life behavior.

The fatty-acid profile is critical for oil quality and assessing substrate susceptibility to oxidation and is commonly determined as fatty-acid methyl esters (FAMES) by gas chromatography with flame-ionization detection (GC-FID). Following the classical base-catalyzed transesterification procedure of Metcalfe and Schmitz (1961), later optimized for edible oils (Lall et al. 2009; Grüneis et al. 2019; Pignitter et al. 2014), oils are extracted into n-hexane and analyzed on polar capillary columns such as ZB-WAX. Quantification relies on

external FAME calibration. Complementary information on relative PUFA depletion can be obtained by  $^1\text{H}$  NMR from the bis-allylic proton region without derivatization (Martínez-Yusta and Guillén 2014). Partial hydrolysis of triacylglycerols and the formation of free fatty acids are routinely monitored by  $^1\text{H}$  NMR through integration of the glycerol backbone and terminal methyl resonances. This approach enables direct estimation of mono- and diacylglycerols and complements the AV determination specified by AOCS (2017).

The antioxidant capacity is often screened using colorimetric assays such as ABTS/TEAC (Re et al. 1999), DPPH (Brand-Williams et al. 1995) or FRAP (Benzie und Strain 1996), which provide comparative but non-specific indices of redox activity. However, cold-pressed oils include several antioxidant types such as tocopherols, polyphenols, carotenoids and chlorophylls. Tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ) are typically analyzed by reversed-phase HPLC with UV or fluorescence detection following the protocol of (Gliszczynska-Swiglo et al. 2007), using rac-tocol as internal standard and a C18 separation. The total phenolic content (TPC) is widely assessed via the Folin–Ciocalteu spectrophotometric assay as described by Singleton und Rossi (1965), while compound-specific phenolics are quantified by LC-MS/MS, mostly in multiple-reaction-monitoring (MRM) mode, employing representative standards for phenolic acids, flavonoids and lignans (Fruehwirth et al. 2020). Untargeted phenolic profiling is achieved by LC-HRMS (QTOF or timsTOF) with software-assisted feature extraction. Chlorophylls and carotenoids are determined spectrophotometrically after cyclohexane extraction, according to (Herchi et al. 2015), from absorbances at approximately 670 nm and 470 nm, respectively.

Primary oxidation products are typically analyzed via the peroxide value (PV), determined by iodometric titration, which still remains the reference measure for LOOHs (Wheeler 1932). It is the only oxidation index with Codex limits. PV is expressed as meq  $\text{O}_2 \text{ kg}^{-1}$  oil and complemented by the measurement of conjugated diene absorption ( $\text{K}_{232} \approx 231\text{--}235 \text{ nm}$ ).  $^1\text{H}$  NMR can further differentiate total LOOHs and hydroperoxy-conjugated dienes by their characteristic proton resonances near 8.3–8.9 ppm (Martínez-Yusta und Guillén 2014). Secondary lipid oxidation products are mainly aldehydes, ketones, alcohols and short-chain acids. Those are typically analyzed by solid-phase microextraction (SPME) coupled to GC-MS. The microextraction concept was introduced by Arthur & Pawliszyn (1990) and subsequently adapted for edible oils using DVB/CAR/PDMS fibers, controlled headspace equilibration and split/splitless thermal desorption (Nieva-Echevarría et al. 2017). Identification

usually relies on spectral matching scores from databases and calibration for key volatiles.

Accelerated shelf-life testing by the Rancimat method remains the standard for rapid comparison of oil stability. The technique, standardized by AOCS (1995), measures the oxidative induction time (OIT) at elevated temperature from the conductivity rise of volatile acids transported into water. OIT values obtained at different temperatures can be used for Arrhenius-type extrapolation to ambient storage (Aktar und Adal 2019), although accuracy depends on matrix composition and test setup. Complementary evaluation under realistic light and oxygen exposure is recommended to better represent household storage conditions.

Together, these methods provide a comprehensive analytical framework to describe the compositional basis, antioxidant protection and oxidative behavior of cold-pressed oils as reported in the literature.

## **2 AIMS AND HYPOTHESES**

As outlined in the Introduction chapter, the oxidative deterioration of PUFA-rich oils limits their nutritional quality, oxidative stability and shelf-life. The susceptibility of these oils to oxidation is governed by a complex interplay of their lipid composition, antioxidant content and external factors such as processing, storage and packaging conditions. To address these challenges, the overall goal of this doctoral thesis was to explore strategies that enhance nutritional quality and the stability of edible oils and provide further mechanistic understanding of oxidative processes through advanced analytical approaches. The work combined applied and fundamental investigations, aiming to promote sustainable oil production and establish oil-type specific markers of oil deterioration.

The research was structured into five complementary topics, each contributing to one or more objectives: (i) increasing the natural antioxidant potential of oils, (ii) extending their oxidative stability and shelf-life, (iii) implementing sustainable and circular approaches to oil production and storage and (iv) advancing mechanistic understanding and prediction of lipid oxidation. Together, these studies should generate a comprehensive understanding that links nutritional composition, processing strategies and lipid oxidation behaviour.

The first aim was to increase the antioxidant content and nutritional quality of cold-pressed oils by exploring approaches capable of enhancing bioactive compounds. Two strategies were explored: seed germination prior to oil extraction and the valorization of press cake particles in cloudy linseed oil obtained by a green extraction method. Germination was expected to activate endogenous enzymatic and metabolic pathways leading to the accumulation of phenolic compounds, tocopherols and pigments in the seeds, which would subsequently be transferred via cold-pressing into the oil. Similarly, retaining fine press cake particles during oil extraction was anticipated to enrich the oil with antioxidants that are normally lost during the clarification or filtering processes. It was hypothesized that both approaches would yield oils with significantly improved antioxidant capacity, especially polyphenol content and thus enhanced oxidative stability compared to conventional cold-pressed oil references. These studies were designed to provide insight into natural, additive-free means of improving oil quality.

The second objective focused on prolonging the oxidative stability and shelf-life of PUFA-rich oils through targeted antioxidant fortification and comprehensive degradation monitoring. The fortification of oils with the aminophospholipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (PE(16:0/18:1)) was investigated as a natural strategy to mitigate oxidation. This compound was chosen for its potential that aminophospholipids may decompose hydroperoxides or react with carbonyl compounds, thereby possibly acting as preventive or reactive antioxidant. It was hypothesized that PE(16:0/18:1) addition would increase OIT under accelerated oxidation, reduce the formation of primary and secondary oxidation products during storage and preserve native antioxidants such as tocopherols. Complementarily, a six months storage experiment across various cold-pressed oils was designed to monitor fatty acid degradation, polyphenol profiles and volatile compounds formation. It was expected that combining these data with chemometric and ML approaches would allow the identification of robust deterioration markers and for predictive models of oil stability.

The next aim was to advance sustainability and circularity in oil processing and packaging. This goal was addressed from two directions. First, the valorization of press cake material through green extraction was expected to minimize waste and create added value by transferring bioactive compounds into the oil, aligning with circular economy principles.

Second, the potential of biodegradable polylactic acid (PLA) as an alternative to conventional polyethylene terephthalate (PET) packaging was assessed under realistic storage conditions. It was hypothesized that PLA would maintain oxidative stability comparable to PET while possibly reducing environmental impact and avoiding migration of non-intentionally added substances (NIAS). These studies collectively sought to establish process and packaging concepts that combine product stability with ecological responsibility.

Beyond applied improvements, the thesis also aimed to deepen the mechanistic understanding of lipid oxidation pathways and antioxidant interactions in complex oil matrices. The progression from primary to secondary oxidation products, the depletion kinetics of antioxidants and the accumulation of primary and secondary lipid oxidation products were examined using a combination of GC-FID, LC-MS/MS, GC-MS and  $^1\text{H}$  NMR. It was hypothesized that the rate and pattern of oxidation differ among oil types depending on their degree of unsaturated fatty acids and antioxidant profiles and that further correlating changes across molecular classes would clarify the sequential reactions occurring during storage. By integrating systematic analytical and statistical approaches, an extended understanding of oxidative mechanisms and their modulation by antioxidants was expected.

Taken together, these projects were designed to provide both practical and theoretical advances in the stabilization of edible oils. The general hypothesis of this thesis is that the oxidative stability of cold-pressed, PUFA-rich oils can be improved and predicted through a combined strategy involving natural antioxidant enhancement, targeted phospholipid fortification, sustainable processing and packaging and integrated degradation biomarker identification. This multidisciplinary approach was expected to bring novel insights into the chemical drivers of oil deterioration, guide the development of stable and high-quality oil products and contribute to sustainable food processing practices.

## **3 PART I: NATURAL & SUSTAINABLE PROCESSES**

### **3.1 RESEARCH SCOPE**

Part I of this thesis focused on natural strategies to enhance the nutritional quality and oxidative stability of cold-pressed vegetable oils, addressing one of the key challenges limiting their broader use and shelf-life. Cold-pressed oils, while valued for their high content of PUFAs and health-promoting bioactives, are inherently prone to rapid oxidation. To overcome this limitation without resorting to synthetic additives or refining, this part explored two complementary, sustainable approaches aimed at increasing the antioxidant potential of oils. To achieve this, seeds were germinated before pressing and bioactive substances were enriched through a green extraction from press cake residues.

The first study investigated whether controlled seed germination could naturally improve the nutritional profile and oxidative stability of linseed and sunflower seed oils. Germination activates endogenous enzymes responsible for the synthesis and release of antioxidant compounds such as phenolic acids, flavonoids and tocopherols, while potentially modifying the lipid fraction and fatty acid composition. It was hypothesized that these biochemical changes could delay lipid oxidation and thereby extend the predicted shelf-life of oils analyzed. The study combined comprehensive lipid and polyphenol profiling with oxidative stability assays to evaluate whether germination represents a viable, natural pretreatment strategy for a novel oil product.

The second study used the concept of a cloudy linseed oil as an outcome of a green extraction process that intentionally retains bioactive-rich press cake particles in the oil matrix. This method aimed to valorize by-products from oil pressing by transferring polyphenols, pigments and other antioxidants from the residual cake into the oil. As a result, improved nutritional values and oxidative stability were expected. Unlike conventional refining or filtration, which remove such particles to obtain clarified oils, this approach exploited their presence as a functional component. The resulting cloudy linseed oils were characterized for their antioxidant composition, polyphenol profile, oxidative induction time and primary and secondary lipid oxidation markers, allowing evaluation of how suspended solids and colloidal structures contribute to enhanced stability and flavor quality.



Together, these studies demonstrate that bioprocessing and upcycling approaches can significantly enhance oil quality and stability without chemical additives. Both germination and green extraction represent sustainable, low-input strategies that align with circular economy principles by improving the quality of plant-based oils and reducing waste from oilseed processing. By elucidating the mechanisms through which naturally occurring antioxidants and particle-associated bioactives influence lipid oxidation, Part I provides new insights into how simple modifications can produce more stable and nutritionally superior cold-pressed oils.

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## 3.2 ORIGINAL WORKS

### 3.2.1 Comprehensive Analysis of Oxidative Stability and Nutritional Values of Germinated Linseed and Sunflower Seed Oils

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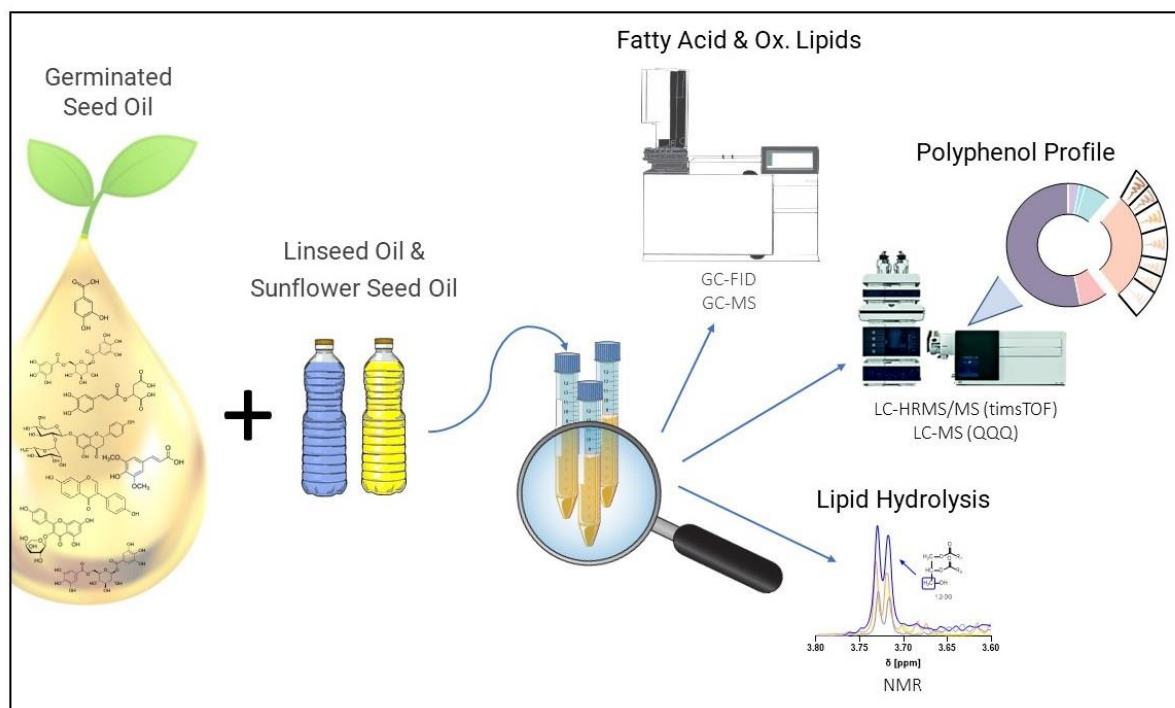
<sup>d</sup> Agrofila, Stip, N. Macedonia

**Keywords:** Germinated oils, oxidative stability, antioxidant capacity, polyphenols, shelf-life

**Status:** Published

**Contribution:** I drafted and optimized the study design and developed the experimental concept, conducted the analytical work, including GC-FID, SPME-GC-MS, LC-MS, HPLC-UV, primary lipid oxidation parameters and antioxidant assays, carried out data curation, statistical evaluation and figure preparation and wrote the original manuscript draft.

## Graphical Abstract



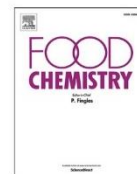
## Highlights

- Germination significantly increased antioxidants in linseed oil and sunflower seed oil
- Specific polyphenol profiles of germinated oils identified for first time using LC-MS
- DG content significantly increased from 1.11 to 3.6 mg/g in germinated linseed oil
- Rancimat predicted  $4.10 \pm 0.52$ -fold longer shelf-life in germinated linseed oil
- Oil stability clearly increased despite GC-MS findings



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

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## 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  $37.2 \pm 3.5$ -fold and  $11.6 \pm 1.5$ -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  $4.10 \pm 0.52$ -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 annuus* 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)  $\alpha$ -linolenic acid (ALA), is noteworthy for its contribution to a balanced omega-6 to omega-3 ratio in the diet (Farag, Elmam, & 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 secoisolaricresinol 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;

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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, Martínez-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, Galkowska, Rożnowski, & Fortuna, 2014; Somsong, Santivarangkna, Tiyyon, 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<sub>2</sub>O), 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, Lorojaronphon, 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<sub>2</sub>O (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<sub>3</sub> solution (20 mM) as FRAP reagent. Standards were prepared using an FeSO<sub>4</sub> × 7H<sub>2</sub>O 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<sub>2</sub> evaporation and the aqueous phase was freeze-dried. The extracted polyphenolic samples were dissolved in 100  $\mu$ L 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  $\mu$ m). 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<sub>2</sub>O 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<sup>2</sup> 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  $\times$  3.0 mm, 5  $\mu$ m, 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<sub>2</sub>) and 10 L/min (N<sub>2</sub>), 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  $\mu$ L of rac-tocol (50 mg/mL) with 900  $\mu$ L of n-hexane. A total of 1  $\mu$ L of the rac-tocol dilution was pipetted into each sample and the  $\alpha$ -,  $\gamma$ -, and  $\delta$ -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  $\mu$ m EVO C18 column (150  $\times$  4.6 mm), at 295 nm using a diode array detector (Dionex DAD-3000), enabling  $\alpha$ -,  $\gamma$ -, and  $\delta$ -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  $\times$ 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 (<sup>1</sup>H NMR)

The <sup>1</sup>H NMR spectra of the samples were acquired using an Avance 400 spectrometer (Bruker, Vienna, Austria) operating at 400 MHz. Briefly, 100  $\mu$ L of the oil sample was dissolved in 500  $\mu$ L 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-5ms 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  $42.22 \pm 0.24\%$ . After germination, the oil content decreased significantly to an average value of  $27.71 \pm 0.21\%$ . Similarly, the oil content of linseed before germination was slightly lower at  $35.14 \pm 0.45\%$  and decreased to a value of  $25.35 \pm 0.3\%$  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  $\alpha$ -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  $\alpha$ -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

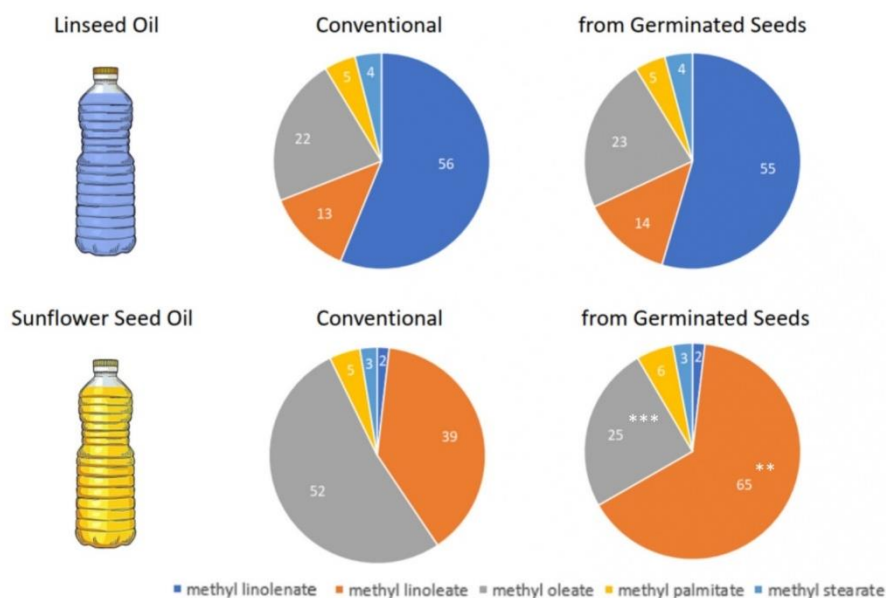


Fig. 1. Fatty acid profiles measured by GC-FID shows the fatty acid distribution of the oils in  $\alpha$ -linolenic acid, linoleic acid, oleic acid, palmitic acid and stearic acid. One-way ANOVA was conducted with Tukey post-hoc test, and data are presented as mean,  $n = 3$ , \*\* =  $p < 0.01$ , \*\*\*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



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 (Commission 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  $5 \pm 1$  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  $572.8 \pm 13.1$  mg/kg and  $139.5 \pm 15.2$  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 ( $236.30 \pm 0.46$  mg GAE/kg) and antioxidant activity (DPPH:  $605.62 \pm 0.39$   $\mu\text{mol TE kg}^{-1}$ , ABTS:  $1864.41 \pm 4.84$   $\mu\text{mol TE kg}^{-1}$ ) 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

**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  $\pm$  SD. n = 3, \*\*\* =  $p < 0.001$ , \*\*\*\* =  $p < 0.0001$ .

| Oil Sample                            | ABTS<br>[ $\mu\text{mol TE/kg}$ ] | DPPH<br>[ $\mu\text{mol TE/kg}$ ] | FRAP<br>[nmol Fe(II)/<br>kg] | TPC<br>[mg GAE/kg]    | Recovery<br>[%] |
|---------------------------------------|-----------------------------------|-----------------------------------|------------------------------|-----------------------|-----------------|
| Conventional<br>Linseed Oil           | $17.9 \pm 1.1$                    | $9 \pm 0.2$                       | $169.9 \pm 12.9$             | $15.4 \pm 1.4$        | 98.7            |
| Germinated<br>Linseed Oil             | $1612.1 \pm 45.3$ ****            | $609.9 \pm 11.4$ ****             | $3092.2 \pm 85$ ****         | $572.8 \pm 13.1$ **** | 106.4           |
| Conventional<br>Sunflower<br>Seed Oil | $10.9 \pm 1.5$                    | $10 \pm 0.1$                      | $139.9 \pm 17.2$             | $12 \pm 1.3$          | 97.3            |
| Germinated<br>Sunflower<br>Seed Oil   | $245 \pm 31.7$ ****               | $177.1 \pm 8.1$ ****              | $590 \pm 42.9$ ***           | $139.5 \pm 15.2$ **** | 105.8           |

ABTS: 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay in  $\mu\text{mol TE/kg}$ , DPPH: 1,1-diphenyl-2-picrylhydrazyl assay in  $\mu\text{mol TE/kg}$ , FRAP: ferric reducing antioxidant potential assay in nmol Fe(II)/kg, TPC: total polyphenol content in mg gallic acid equivalents (GAE)/kg, Recovery of GAE in %.

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.

#### 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 phytochemicals, 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, plastochochromanol-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). Plastochochromanol-8 is mainly found in linseeds and has more pronounced antioxidant properties than  $\alpha$ -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

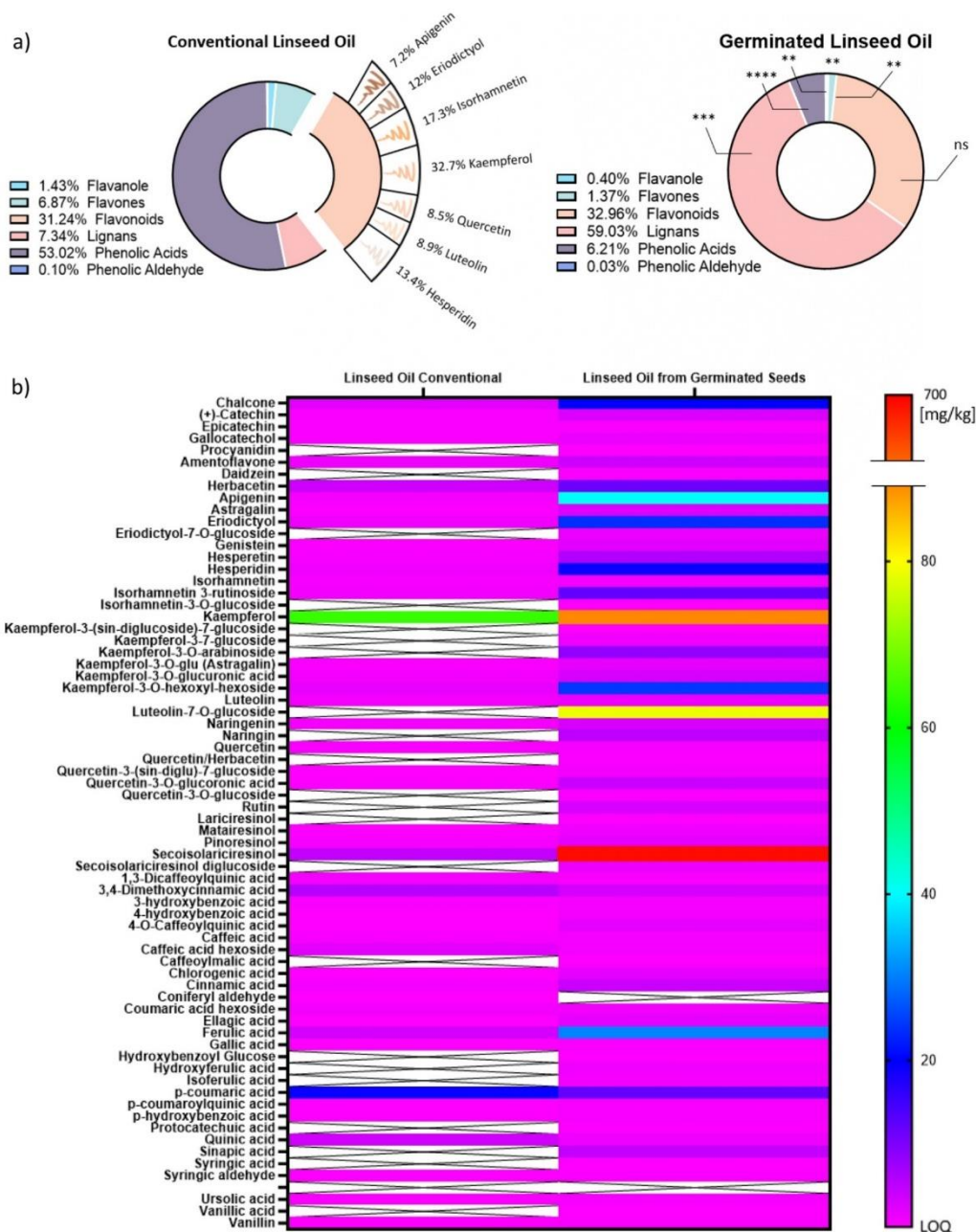


research is required to elucidate the nutritional and functional properties of these oils.

### 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

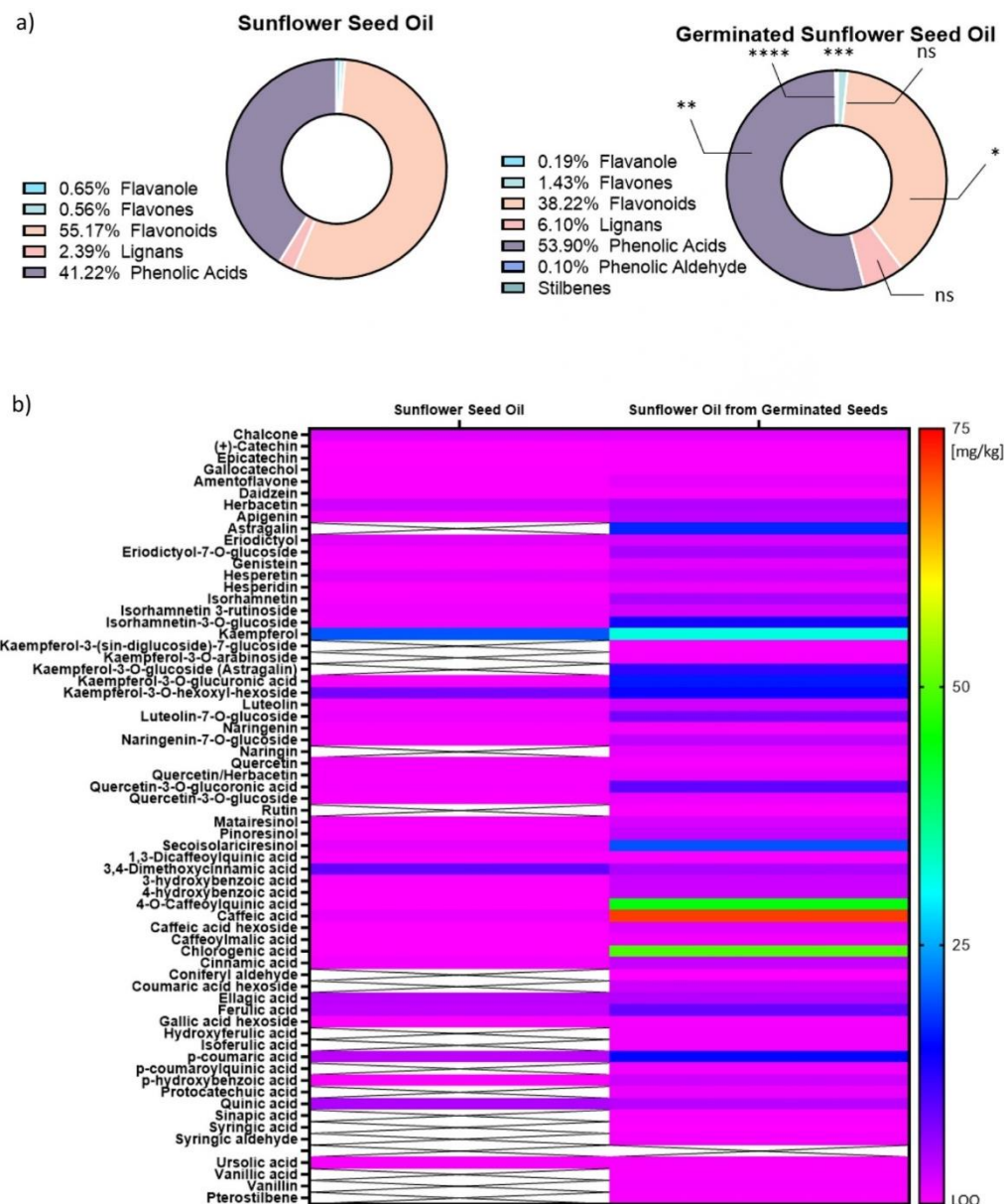
into specific categories including flavanols, flavones, flavonoids, lignans, phenolic acids, phenolic aldehydes, and stilbenoids. Comprehensive analysis revealed a substantial increase in the number of quantified polyphenols from germinated seed oils compared to conventional oils, with linseed oil showing 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  $0.12 \pm 0.01$  mmol/kg, while germinated linseed oil showed  $2.28 \pm 0.34$  mmol/kg. In comparison, sunflower seed oil contained  $0.1 \pm 0.01$  mmol/kg, and germinated sunflower seed oil showed  $0.95 \pm 0.08$  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 ( $9.78 \pm 1.73$  mg/kg), followed by p-coumaric acid ( $19.17 \pm 1.9$  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  $< \text{LOD}$  and  $< \text{LOQ}$ . (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 ( $650.53 \pm 112$  mg/kg, 142.3-fold change), kaempferol ( $102.3 \pm 14.31$  mg/kg, 10.46-fold change), and luteolin-7-O-glucoside ( $78.62 \pm 4.5$  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 ( $16.67 \pm 1.9$  mg/kg) and 3,4-dimethoxycinnamic acid ( $9 \pm 1.49$  mg/kg), while germinated sunflower seed oil exhibited a substantial rise in caffeic acid ( $71.41 \pm 7.66$  mg/kg, 72.23-fold increase), chlorogenic acid ( $49.34 \pm 8.12$  mg/kg), and 4-O-caffeoylquinic acid ( $44.38 \pm 5.9$  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-hexosyl-hexoside ( $+6.25$  mg/kg). Additionally, specific polyphenolic compounds were found exclusively in one oil or the other, such as astragalol 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  $61.1 \pm 4.9$  mg/100 g oil contained significantly higher values than linseed oil, with  $32.8 \pm 1.6$  mg/100 g. Consistent with the observations of Zhang et al., 2023, linseed oil displayed a predominance of  $\gamma$ -tocopherol, with discernible increases in  $\alpha$ -tocopherol and  $\delta$ -tocopherol levels after the germination process. In contrast, conventional linseed oil lacks  $\delta$ -tocopherol entirely.

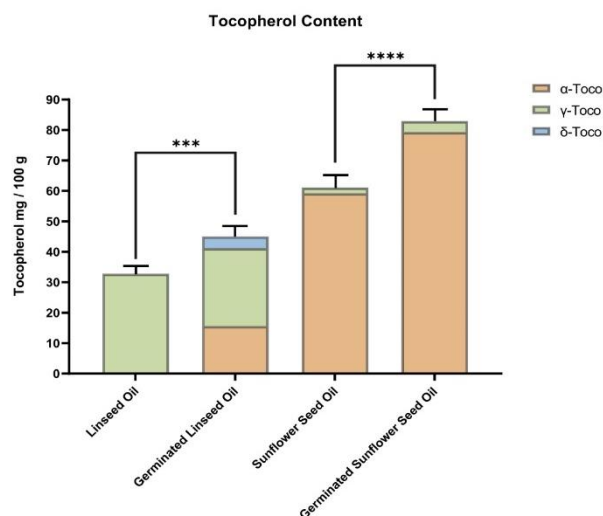


Fig. 4. Tocopherol profiles of oils by HPLC/UV with  $\alpha$ ,  $\gamma$  and  $\delta$ -tocopherol detection. Unpaired t-tests, utilizing parametric tests with a FDR approach for multiple comparisons were conducted, and data are presented as mean  $\pm$  SD in mg tocopherol/100 g oil,  $n = 3$ , \*\*\*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

Conversely,  $\alpha$ -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  $\gamma$ -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  $21 \pm 0.03$  mg/kg. Linseed oil showed a pronounced increase from  $0.61$  to  $16.4 \pm 0.02$  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 ( $15.7 \pm 0.02$  mg/kg) compared to sunflower seed oil, with a content of  $14.1 \pm 0.02$  mg/kg, suggesting distinct phytonutrient transformations 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  $17.5 \pm 0.5$  to  $6 \pm 0.4$  mEq/kg, while in sunflower seed oil, decreased from  $14 \pm 0.7$  to  $8.4 \pm 0.2$  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  $37.2 \pm 0.2$  mg KOH/g, and in germinated sunflower seed oil, it increased from  $3.3$  to  $15.1 \pm 0.3$  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.

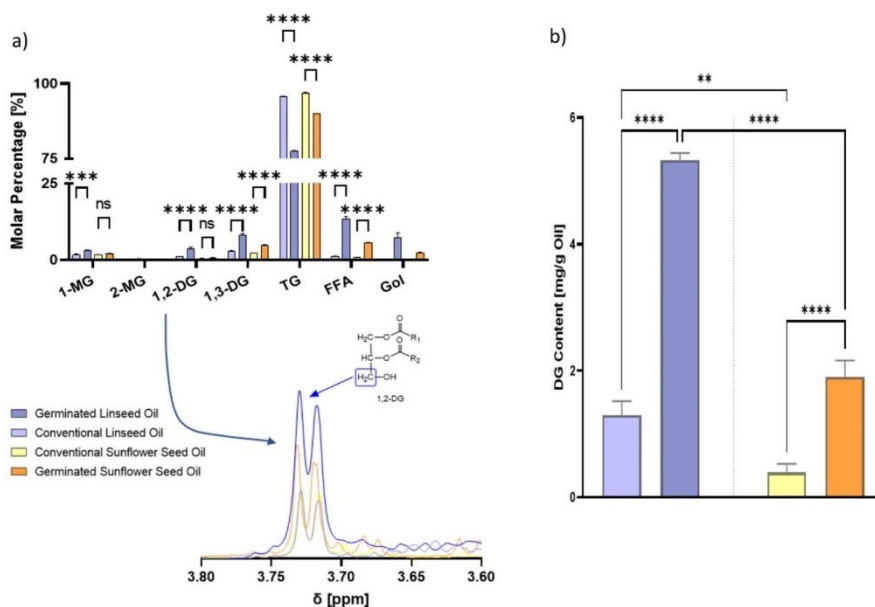
During germination, free fatty acid content in seeds can increase significantly, as observed in flax seeds by Wanasundara, Wanasundara, & Shahidi, 1999. The calculation of free fatty acids (FFA) from both the acid value and NMR results revealed consistent trends in our samples, as indicated by a high 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  $18.44 \pm 0.1\%$  for germinated linseed oil. NMR analysis showed a similar trend, with mean FFA values of  $1.12 \pm 0.1\%$  for conventional linseed oil and substantially higher values of  $13.46 \pm 0.6\%$  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 (Go). Significant decreases in TG content after germination were observed, with linseed oil dropping from  $95.62 \pm 0.17$  to  $77.49 \pm 0.22\%$  and sunflower seed oil from  $96.88 \pm 0.24$  to  $89.9 \pm 0.06\%$ . 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  $1.11 \pm 0.1$  mg/g to  $3.6 \pm 0.62$  mg/g and for sunflower seed oil from  $0.42 \pm 0.19$  mg/g to  $0.74 \pm 0.09$  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 (Go) 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  $\pm$  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  $2.18 \pm 0.05$  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  $4.13 \pm 0.25$ -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  $6.42 \pm 0.06$  h for conventional sunflower seed oil. Shelf-life predictions based on the Arrhenius equation revealed extended shelf-lives for germinated linseed oil ( $10.93 \pm 0.8$  months) and germinated sunflower seed oil ( $9.93 \pm 0.53$  months), compared to conventional linseed oil ( $2.66 \pm 0.22$  months) and sunflower seed oil ( $8.48 \pm 0.37$  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

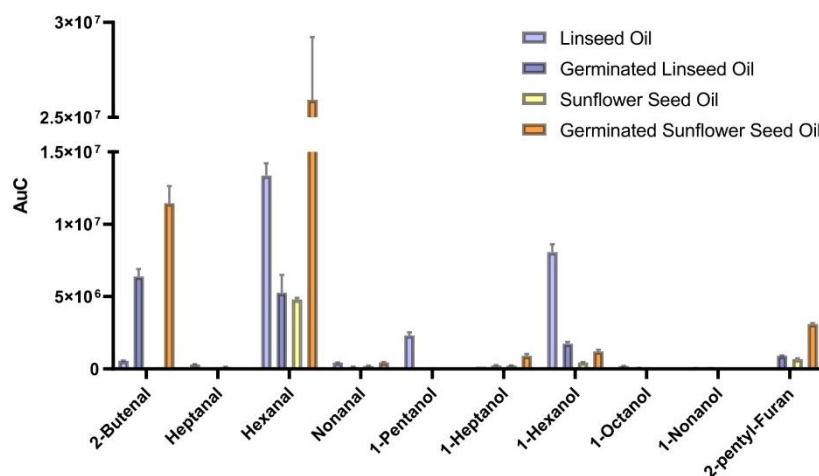


Fig. 6. Analysis of secondary lipid oxidation products by GC-MS analysis revealed drastic alterations in the studied oils. Data are presented as mean  $\pm$  SD,  $n = 3$ .



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 Auñón-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.

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#### 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 ( $\alpha$ -,  $\beta$  +  $\gamma$ - and  $\delta$ -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., Martínez Villaluenga, C., Amigo, L., & Frías, 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.
- Commission 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., Elinani, 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., Klinkeson, 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., Quérou, A., Rombaut, N., Savoie, R., Molinié, R., Thomasset, B., Mesnard, F., & Lainé, E. (2017). The lignan (+)-secoisolaricresinol 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., Auñón-Lopez, A., Pointner, T., Martínez-Yusta, A., König, J., & Pignitter, M. (2023). Poly(lactic 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., Ścisłowski, 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, secoisolariciresinol 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). Pluronic 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., & Fregapani, 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 <sup>1</sup>H 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 annuum* L.)-A review.
- Nieva-Echevarría, B., Encarnación, G., María J., M., & María D., G. (2017). <sup>1</sup>H 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.
- Pajak, 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.
- Petrari, 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., Dunhart, 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., Oliveira 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., Penas, 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). Desinfection of seed surface with sodium hypochlorite. *Phytopathology*, 76, 745–749.
- Schmid, R., Heuckeroth, S., Korf, A., Smirnov, A., Myers, O., Dyrland, T. S., ... Pluskal, T. (2023). Integrative analysis of multimodal mass spectrometry data in MZmine 3. *Nature Biotechnology*, 41, 447–449.
- Sheeba, K., Saunika, 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., Tiayon, 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  $\gamma$ -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 autooxidative 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 autooxidation 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.

### 3.2.2 Enhancing Nutritional Quality and Oxidative Stability in Cloudy Linseed Oil through Green Extraction from Press Cake

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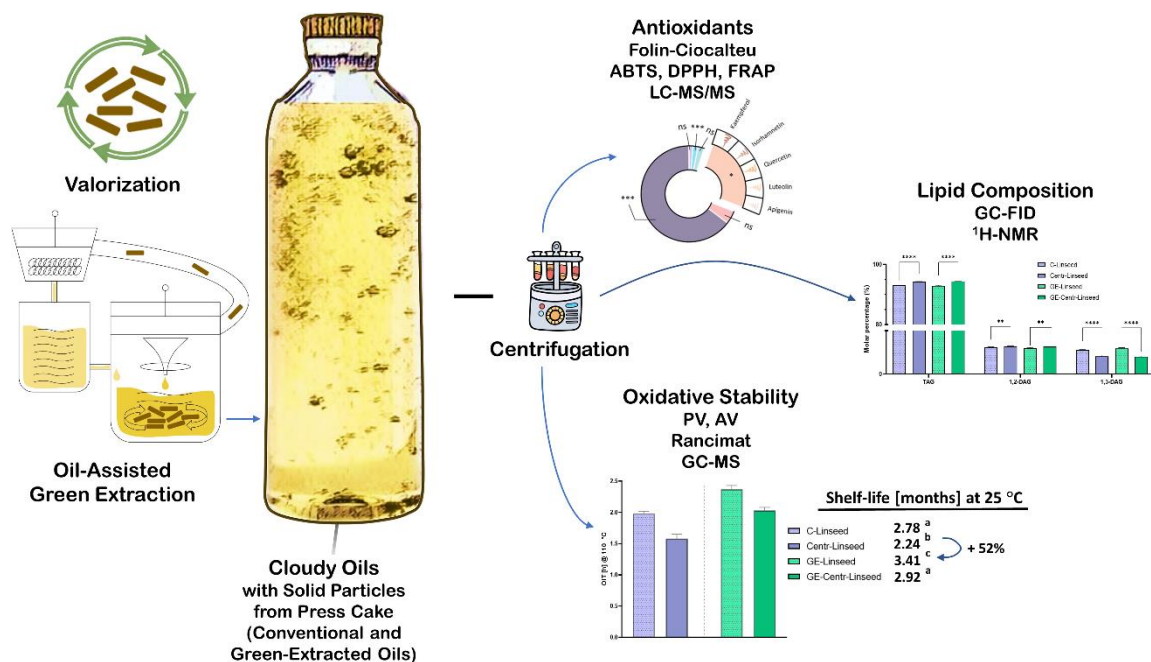
**Keywords:** Cloudy linseed oil, Green extraction, Press cake particles, Oxidative stability, Polyphenols, Shelf-life

**Status:** Published

**Contribution:** I conceptualized and designed the study, carried out the green extraction, performed analytical experiments including GC-FID, GC-MS, LC-MS/MS, HPLC-UV and spectrophotometric assays. I carried out the data processing, statistical analysis and visualization, interpreted results and wrote the original manuscript.



## Graphical Abstract



## Highlights

- Valorization of cloudy linseed oil with press cake particles was investigated
- Green extraction further increased polyphenol content by 530%
- LC-MS/MS identified up to 51 polyphenols, linked to press cake turbidity
- Oxidative induction time improved by 52% compared to clear linseed oil
- Green extraction decreased hexanal by 93%, while alcohols like 1-octanol increased



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### ABSTRACT

In response to increasing consumer demand for sustainable and health-conscious food choices, this study explored the potential of cloudy linseed oil generated through a modified oil-assisted green extraction process. Our approach aimed to retain bioactive-rich press cake particles and maximize their antioxidant potential. Cloudy linseed oils after green extraction showed significantly increased polyphenol content by 530 % and improved antioxidant capacities. LC-MS/MS analysis discovered the distinct effects of centrifugation and green extraction on polyphenol profiles. Up to 51 different polyphenolic substances were quantified. These included flavonoids, lignans, and phenolic acids strongly associated with the presence of particles. This enrichment in antioxidants further enhanced the oxidative induction time measured by Rancimat by 52 % from 2.24 to 3.41 months compared to conventional linseed oil. SPME/GC-MS analysis showed a 93 % reduction in hexanal, a key oxidation marker, while selectively increasing alcohol levels of 1-hexanol and 1-octanol, which may positively influence the flavor profile of cloudy linseed oil. By achieving press cake valorization and enhanced oxidative stability, this strategy offers a sustainable and functional oil product with higher nutritional quality. These findings introduce a novel approach to cold-pressed linseed oil enhancement, distinct from previous oil-assisted green extraction methods focused solely on the clarified oil phase.

### 1. Introduction

*Linum usitatissimum* L., commonly known as flax, is a rich source of oil (known as linseed oil) with high contents of  $\alpha$ -linolenic acid (ALA), comprising nearly 60 % of its fatty acid composition. Clicken oder tippen Sie hier, um Text einzugeben. This essential omega-3 fatty acid contributes to the oil's potential health benefits, including lowering cholesterol levels and improving blood glucose responses (Haque et al., 2024). Its fatty acid profile also contains notable amounts of oleic and linoleic acids (Shim et al., 2015). This composition makes linseed oil a valuable addition to other vegetable oils, as it can help balance the dietary omega-6 to omega-3 fatty acid ratio (Joshi et al., 2022). Its nutritional quality is further complemented by various bioactive compounds, including tocopherols, carotenoids, and chlorophyll (Obranović et al., 2015). Volatile compounds in linseed oil include aldehydes and alcohols, which contribute to its flavor (Sun et al., 2023). These properties make cold-pressed linseed oil a nutritious and health-promoting option, ideal for functional foods or everyday edible oil (Rabiej-Kozioł et al., 2023).

However, its high polyunsaturated fatty acids (PUFA) content makes it highly susceptible to lipid oxidation. Strategies to enhance its stability

include antioxidant fortification and production process modifications (Grosshagauer et al., 2019). The stability of linseed oils can be evaluated through various methods, and their antioxidant activity can be a key factor (Gülmez & Şahin, 2019). Polyphenols are bioactive, natural antioxidants found in plants that play a crucial role in protecting oils from oxidative degradation and improving their overall stability (Pointner et al., 2024; Zhang et al., 2023). The polyphenolic content can vary, with cold-pressed linseed oil having 1.82–34.21 mg polyphenols per kg (Kostadinović Velickovska et al., 2018). Polyphenols can be divided into flavonoids, lignans, phenolic acids, stilbenoids and tannins (Zeb, 2021). Recent studies have explored innovative methods to enhance polyphenol content in cold-pressed oils and by-products, to improve stability and health functionality. Techniques such as ultrasound-assisted extraction and pulsed electric fields (PEF) have shown promising results in improving yield and antioxidant activity of polyphenols from flaxseed cake (Drévillon et al., 2023; Kaur et al., 2024). Fruehwirth et al. (2020) developed a green extraction process by using linseed oil as an extraction medium for polyphenols from press cake. Their method involved repeated mixing and centrifugation cycles to transfer hydrophilic antioxidants into the oil phase, resulting in a 12-fold increase in polyphenol concentration and a 38 % improvement in oxidative stability

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compared to untreated oil.

If oils are not centrifuged or filtered after cold-pressing, particles from the press cake can remain in the oil, potentially affecting its quality and stability. These oils can have a higher polyphenolic content, nutritional value and different flavor and aroma due to a greater amount of tocopherols, carotenoids, and phospholipids compared to refined oils (Chandra et al., 2020). For instance, Tsimidou et al. (2005) found that cloudy olive oils have higher stability due to the individual polyphenols released during hydrolytic processes. Fittingly, Bendini et al., (2013) also showed that cloudy oils such as non-filtered extra virgin olive oil (EVOO) can retain high levels of bioactive compounds. The cloudiness in these oils is due to the presence of water micro-droplets and solid particles, as well as natural emulsifiers (Koidis et al., 2008). These oils can be stable under oxidizing conditions, with the turbidity disappearing over time due to chemical bonding between components (Lercker et al., 1994). Recent work on veiled extra virgin olive oil (VVOO) has shown that the colloidal dispersion of suspended particles, polar phenolics, and microdroplets of water may contribute to enhanced oxidative stability via interfacial interactions between the lipid phase and bioactive compounds (Cinelli et al., 2020). This colloid-based stabilization mechanism has not yet been explored in linseed oils.

While cloudy olive oils have been studied extensively, to the best of our knowledge, no prior work has investigated cloudy linseed oils with retained press cake particles. This represents a critical gap in the literature, particularly given the growing interest in unrefined, functional oil. The aim of this study was therefore not only to enhance the nutritional profile and oxidative stability of linseed oil through green extraction, but to evaluate for the first time the impact of suspended bioactive-rich press cake particles on oil quality, shelf-life, and composition. Unlike Fruehwirth et al. (2020), who allowed particles to sediment and focused on the clear oil phase, our work explicitly investigates cloudy oils as a novel product with similar benefits as non-filtered EVOO. We hypothesize that cloudy linseed oils can exhibit enhanced shelf-life and nutritional value. Hence, this study investigated how green extraction and controlled centrifugation affect antioxidant enrichment, oxidative stability and lipid composition. By preserving bioactive compounds typically lost during refining, this method could offer a new balance between nutritional functionality and consumer acceptance. In addition, this strategy might contribute to sustainable food processing by upcycling press cake into a value-added functional component, supporting circular economy principles.

## 2. Materials and methods

### 2.1. Chemicals and materials

All chemicals were of analytical or LC-MS grade and purchased from Sigma-Aldrich (Vienna, Austria), Carl Roth (Karlsruhe, Germany), and VWR International GmbH (Vienna, Austria). A detailed list of all chemicals used is provided in the Supplementary Material.

### 2.2. Oil samples

Conventional, cloudy, non-filtered, cold-pressed linseed oils and the remaining press cake were purchased from the organic supplier Hagenthaler Ölmanufaktur (St. Andrä-Wördern, Austria). This oil (C-Linseed oil) was analyzed in its original form and served as basis for the other samples. It was either centrifuged to remove suspended solids (Centr-Linseed oil) or subjected to green extraction using linseed press cake to yield a polyphenol-enriched version (GE-C-Linseed oil), which was subsequently centrifuged (GE-Centr-Linseed oil). For clarity, the following acronyms will be used throughout the manuscript: C-Linseed oil (cloudy, non-filtered linseed oil, as purchased), Centr-Linseed oil (centrifuged, clear linseed oil), GE-C-Linseed oil (green-extracted, cloudy linseed oil), and GE-Centr-Linseed oil (green-extracted, centrifuged, clear linseed oil). The samples analyzed are shown in Fig. S1.

### 2.3. Green extraction and centrifugation

Green extraction was based on Fruehwirth et al. (2020) with their optimized conditions and slight modifications to further improve the antioxidant content of linseed oils. Briefly, a three-stage extraction from flaxseed press cake with oil was carried out. The press cake was ground for 2 min (Büchi mixer B-400) and 15 g were mixed with 30 mL C-Linseed oil and stirred at room temperature for 30 min. The mixture was centrifuged at 3200×g, 4 °C for 5 min, and the supernatant was collected. Extraction was repeated two more times and the remaining cloudy oil phases were combined to increase polyphenol transfer. One batch of each cloudy linseed oil (conventional and green-extracted) was then centrifuged three additional times under the same conditions to remove residual press cake particles. For the oils without additional centrifugation (cloudy oils), particles were not left to sediment prior to analysis, unlike the method of Fruehwirth et al. (2020). Oil samples (cloudy and filtered) were purged with argon and stored at −80 °C.

To determine the solid content, 10 g oil were centrifuged three times at 3200×g, 4 °C for 5 min, and clear phases were removed. The remaining pellet was washed three times with 5 mL *n*-hexane, vortexed 30 s, centrifuged, and the *n*-hexane discarded. The pellet was dried under N<sub>2</sub>, weighed, and the solid content calculated (g/L oil).

### 2.4. Antioxidants

#### 2.4.1. Chlorophyll and carotenoids

Chlorophyll and carotenoids were analyzed following the method of Herchi et al. (2015). For sample preparation, 1.5 g sample were weighed into 15 mL tubes, and 5 mL cyclohexane were added. The mixture was vortexed and centrifuged at 2280×g, 4 °C for 6 min. The chlorophyll and carotenoid contents were then measured at 670 nm and 470 nm, respectively, using a Spark spectrophotometer (Tecan Group Ltd., Switzerland).

#### 2.4.2. Tocopherols by high-performance liquid chromatography with ultraviolet detection (HPLC/UV)

Tocopherols were determined using a method based on Gliszczynska-Swiglo et al. (2007) with slight modifications from Pignitter et al. (2019). In brief, samples were extracted with 2-propanol, spiked with rac-tocol as internal standard, and analyzed by HPLC/UV at 295 nm.  $\alpha$ - and  $\gamma$ -tocopherol were quantified. Full details, including standard preparation, chromatographic conditions, and calibration curves (Fig. S2), are provided in the Supplementary Information.

#### 2.4.3. Total polyphenol content and antioxidant capacities

Detailed protocols for the extraction of polyphenolic compounds, the Folin-Ciocalteu assay for total polyphenolic content (TPC), and antioxidant capacities are provided in the Supplementaries. Briefly, polyphenols were extracted by using 2.5 g oil dissolved in 2.5 mL *n*-hexane and 80 % methanol (v/v) following Singleton et al. (1999). After vortexing and centrifugation, the polar phase was recovered, and this process was repeated three times to maximize recovery. The combined extracts were washed with *n*-hexane to remove residual lipids. TPC was measured using the Folin-Ciocalteu assay at 765 nm and expressed as mg gallic acid equivalents (GAE)/kg. The recovery [%] was determined by spiking oil samples with known amount of gallic acid standard prior to extraction and comparing the measured TPC to the theoretical value. Antioxidant capacities were evaluated using the 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Re et al., 1999), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Brand-Williams et al., 1995), and Ferric Reducing Antioxidant Power (FRAP) assays (Benzie & Strain, 1996), with results expressed as  $\mu$ mol Trolox equivalent (TE)/kg or  $\mu$ mol Fe (II)/kg oil for FRAP. The standard curves are shown in Fig. S3-4.



#### 2.4.4. Quantification of polyphenols by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

The protocol from [Pointner et al. \(2024\)](#) was followed for the quantification of polyphenols using targeted LC-MS/MS. Polyphenol extraction involved dissolving 3.5 g of each of the four analyzed oils in 3.5 mL *n*-hexane in a 15 mL centrifuge tube to remove lipids, following the procedure outlined in section 2.4.3. Extracted polyphenolic samples were reconstituted in 100  $\mu$ L 80 % methanol (v/v) and passed through 0.22  $\mu$ m PVDF filters in vials before injection into a LCMS 8040 system with triple quadrupole and ESI source (Shimadzu, Korneuburg, Austria). Separation was conducted on a C18 column (Kinetex, 150  $\times$  2.1 mm, 2.6  $\mu$ m, Phenomenex, Aschaffenburg, Germany) with a flow rate of 0.3 mL/min. Water with 0.1 % formic acid (A) and acetonitrile with 0.1 % formic acid (B) were used as mobile phases. The gradient program for solvent B started at 10 %, for 2 min, increased to 80 % by 8 min, reaching 100 % at 20 min and back to 10 % at 21.1 min, until the end at 25 min. Measurements were operated in negative and positive multiple reaction monitoring (MRM) mode using LabSolutions software with a collision energy (CE) of 30 and -30 V. Declustering potentials (DP), as well as desolvation line, Q-array, and pre-rod biases were managed via standard values recommended by the manufacturer. Polyphenolic compounds were categorized into flavanols, flavones, flavonoids, lignans, phenolic acids, phenolic aldehydes, and stilbenoids. Representative standards, namely kaempferol, quercetin-3-glucoside, secoisolariciresinol, caffeic acid, ferulic acid, p-coumaric acid, gallic acid, and resveratrol, were used for quantification (Table S1). Limits of detection (LOD) and quantification (LOQ) were determined using signal-to-noise ratios (S/N) of 3 and 10, respectively. Data processing, including peak picking, alignment, and quantification, was performed using Skyline v23.0 software to accurately characterize the specific polyphenols, as described by [Pino et al. \(2020\)](#).

#### 2.5. Oxidative stability and shelf-life prediction

##### 2.5.1. Peroxide and acid value determination

The peroxide value (PV), indicator of primary oxidation products, was measured using a titrimetric method based on [Wheeler \(1932\)](#). Oil samples were mixed with an acetic acid:chloroform 3:2 (v/v) solution and potassium iodide, followed by titration with 50 mmol/L sodium thiosulfate until discoloration occurred.

The acid value (AV) was determined following [ISO 660:2020 \(2020\)](#), where oil samples were dissolved in an ethanol/diethyl ether 1:1 mixture (v/v), heated, and titrated with 50 mmol/L potassium hydroxide (KOH) in the presence of seven drops of phenolphthalein. Full protocols, reagent details, and calculation methods are available in the Supplementary Information.

##### 2.5.2. Rancimat analysis

The oxidative stability was assessed using the Rancimat method, following the protocol of [Aktar and Adal \(2019\)](#). This method evaluates the stability of oils by measuring the oxidative induction time (OIT), utilizing a Rancimat apparatus (Metrohm 892, Herisau, Switzerland). For each analysis, 3 g oil were placed in vessels ( $\varnothing$  24  $\times$  150 mm) with an airflow rate of 20 L/h. The OIT of the linseed oil samples was measured at temperatures of 100, 110, and 120 °C. The resulting data were used to predict shelf-life by applying the Arrhenius equation:

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

where A is the pre-exponential factor (frequency factor),  $E_a$  is the activation energy in kJ/mol, R is the gas constant in kJ/(mol·K), and T is the temperature in K.

#### 2.6. Lipid composition

##### 2.6.1. Analysis of fatty acid profiles by gas chromatography-flame ionization detection (GC-FID)

Fatty acid profiles of the oil samples were determined by analyzing their respective fatty acid methyl esters (FAME) using GC-FID (GC-2010 Plus, Shimadzu, Korneuburg, Austria). Extraction and transesterification were performed following a well-established method based on [Pignitter et al. \(2014\)](#). In brief, 100 mg oil were mixed with a heptadecanoic acid methyl ester (HME) internal standard (w/w), toluene, pyrogallol, and 0.5 mol/L sodium methoxide. Liquid-liquid extraction was carried out using *n*-hexane, after which the upper organic phase was collected, dried with sodium sulfate, and filtered through 0.45  $\mu$ m PVDF filters, prior to GC-FID analysis. Standard curves are shown in Table S2.

##### 2.6.2. Determination of hydrolysis products by proton nuclear magnetic resonance ( $^1\text{H}$ NMR)

The hydrolysis products in the oil samples were analyzed using  $^1\text{H}$  NMR spectroscopy, performed on an Avance 400 spectrometer (Bruker, Vienna, Austria) operating at 400 MHz. 100  $\mu$ L oil samples were dissolved in 500  $\mu$ L of deuterated chloroform containing 0.2 % non-deuterated chloroform and 0.03 % tetramethylsilane as internal reference. The acquisition parameters were: 6410 Hz spectral width, 3 s relaxation delay, 128 scans, 4.819 s acquisition time, and 90° pulse width. The spectra were processed and analyzed using MestReNova 14.0 software. Identification and quantification of glycerol structures in molar percentages were conducted according to [Nieva-Echevarría et al. \(2014\)](#).

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

Secondary lipid oxidation products were analyzed according to [Holler et al. \(2023\)](#). A total of 2.5 g 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 (50/30  $\mu$ m, 1 cm length; Supelco, Bellefonte, PA, USA), preconditioned at 250 °C for 30 min. Samples underwent a 5 min pre-equilibration at 50 °C before headspace extraction for 55 min. The fiber was desorbed in the injector port at 250 °C for 10 min in splitless mode, with a 5 min purge time. The carrier gas was helium. Extracted volatiles were analyzed by GC-MS (GC-QP2010 Ultra, Shimadzu, Korneuburg, Austria) equipped with a Zebron ZB-5 ms column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m; Phenomenex). Mass spectra were acquired in full scan mode (*m/z* 40–350) with an electron impact ionization energy of 70 eV. At the start of measurements, the oven was kept at 50 °C for 5 min and then heated by 4 °C per min up to 300 °C and held for 30 min. Compound identification was carried out by matching mass spectra with the NIST library accepting substances with identity score >85 %, and semi-quantification was achieved using arbitrary units based on the area counts of base peak ions.

#### 2.7. Statistical analysis

Statistical analyses were conducted using GraphPad Prism 10.1.1. Normality of the data was assessed using the Shapiro-Wilk test, and equality of variances was evaluated with Levene's test prior to performing one-way ANOVA. Depending on the data set, Tukey's or Šídák's post-hoc test was used for multiple comparisons. Additionally, unpaired t-tests with multiple comparison adjustments using the false discovery rate (FDR) method were performed. Results were considered statistically significant at p-value <0.05. All experiments were conducted with a sample size of  $n \geq 3$ .

### 3. Results and discussion

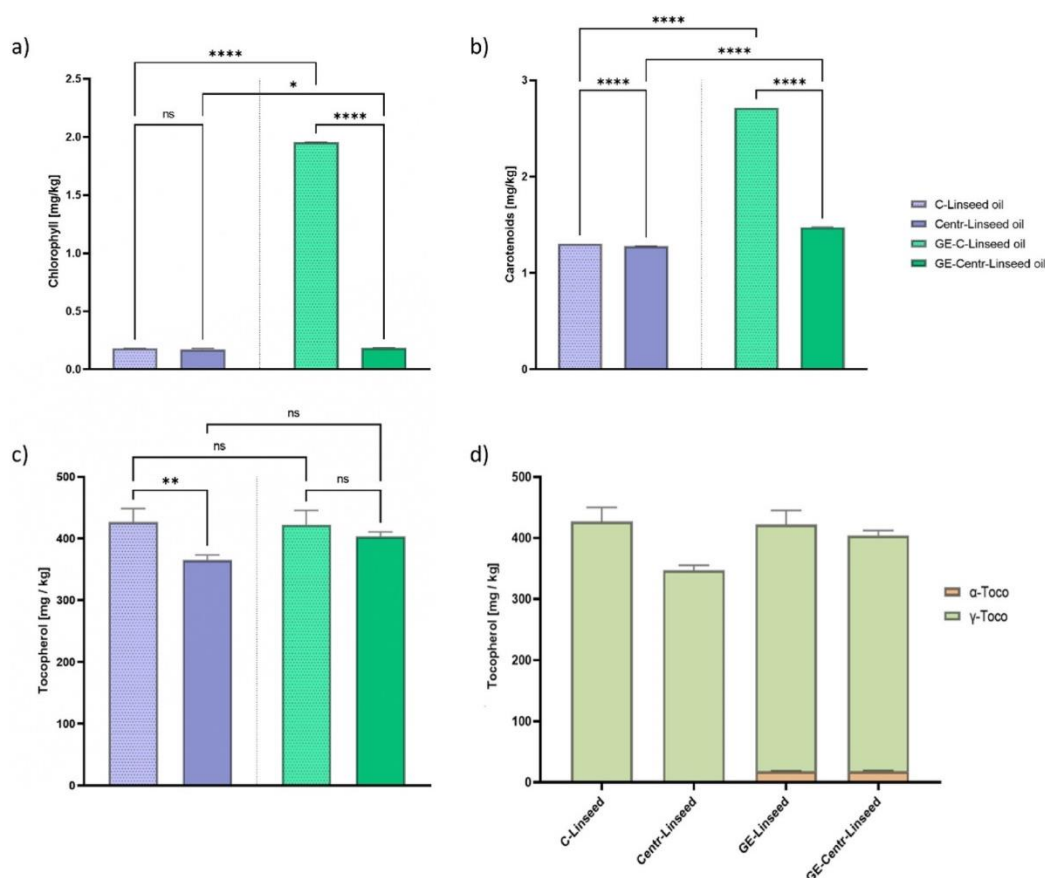
#### 3.1. Antioxidants

##### 3.1.1. Press cake particle, chlorophyll and carotenoid content

The presence of suspended press cake particles in the linseed oils might play a crucial role in their antioxidant composition. To assess the impact of green extraction and centrifugation, the solid particle content was quantified as mentioned in chapter 2.3. The amount of press cake particles in the different linseed oil samples varied significantly depending on the processing method (Table S3). Cloudy linseed oil (C-Linseed oil) contained  $4.0 \pm 0.7$  g/L of suspended solids, which were largely removed after triple centrifugation, reducing the particle content in the clear Centr-Linseed oil to  $0.3 \pm 0.0$  g/L. Green extraction substantially increased the particulate content, as GE-C-Linseed oil contained  $33.3 \pm 3.9$  g/L, indicating a strong retention of press cake material. However, subsequent centrifugation effectively eliminated these particles, with GE-Centr-Linseed oil containing only  $0.4 \pm 0.1$  g/L, confirming that press cake residues were nearly completely removed.

Due to visible color changes in the oils after centrifugation and green extraction, chlorophyll and carotenoid levels were analyzed, as these lipid-soluble pigments not only influence the visual appearance but also contribute to antioxidant properties (Obranović et al., 2015). The impact of green extraction and centrifugation was particularly evident, especially in the cloudy oils, where color differences became more

pronounced, as depicted in Fig. S1. Chlorophyll and carotenoid concentrations are presented in Fig. 1a–b. C-Linseed (0.18 mg/kg) and Centr-Linseed oils (0.17 mg/kg) showed low chlorophyll mean values, while green extraction significantly increased chlorophyll content in GE-C-Linseed oil by a mean 10.9-fold increase to 1.96 mg/kg ( $p < 0.0001$ ). Subsequent centrifugation reduced chlorophyll in GE-Centr-Linseed oil back to 0.18 mg/kg, matching initial samples. A similar trend was observed for carotenoids, where green extraction showed a mean 2.1-fold rise in cloudy GE-C-Linseed oil to 2.72 mg/kg ( $p < 0.0001$ ). Centrifugation after green extraction again reduced carotenoid levels to baseline values. These findings highlight the role of press cake particles in enriching pigment content during green extraction, with centrifugation effectively removing suspended solids and their associated pigments. Compared to values reported by Obranović et al. (2015) where cold-pressed linseed oil contained  $0.35 \pm 0.01$  mg/kg chlorophyll and  $1.78 \pm 0.01$  mg/kg carotenoids, our C-Linseed and Centr-Linseed oils exhibited slightly lower chlorophyll but similar carotenoid levels. The observed color differences post-centrifugation, particularly in GE-Centr-Linseed oil, align with previous studies showing that filtration and refining reduce bioactive compounds in vegetable oils (Bendini et al., 2013; Tsimidou et al., 2005). However, it should be noticed that color differences may not only be explained by the pigment decrease during centrifugation but also by a reduction of turbidity associated with the solid particles loss.



**Fig. 1.** Changes in antioxidant concentrations were analyzed in mg/kg oil. a) showing chlorophyll levels, b) carotenoids in the different linseed oil samples, c) total tocopherol content measured by HPLC-UV, d) stacked bar charts of  $\alpha$ - and  $\gamma$ -tocopherol. One-way ANOVA was conducted, with Tukey post-hoc test, and data are presented as mean + SD,  $n = 3$ , ns = no significance, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.0001$ .



### 3.1.2. Tocopherols by HPLC/UV

The content of tocopherols, markers of vitamin E activity as key lipid-soluble antioxidants, were quantified in the linseed oil samples (Chandra et al., 2020). As shown in Fig. 1c, the total tocopherol content varied slightly among the different processing methods. The initial sample C-Linseed oil contained the highest tocopherol concentration with  $425 \pm 23$  mg/kg oil, which decreased significantly following centrifugation in Centr-Linseed oil to  $365 \pm 21$  mg/kg oil ( $p < 0.01$ ). Interestingly, green extraction maintained tocopherol levels similar to those in C-Linseed oil, and no significant differences were observed. When separated by tocopherol type in Fig. 1d,  $\gamma$ -tocopherol was found to be the dominant form in all samples, in line with previous results from Shadyro et al. (2020) with 494–586 mg/kg  $\alpha$ -tocopherol was only detectable in minor amounts after green extraction. These results indicate that while centrifugation reduces tocopherols in conventional linseed oil, green extraction preserves them, maintaining tocopherol levels even after centrifugation. This aligns with previous observations in non-filtered oils, where suspended solids contribute to the retention of minor bioactive compounds (Koidis et al., 2008).

### 3.1.3. Total polyphenol content (TPC) and antioxidant capacities

The total polyphenol content (TPC) and antioxidant capacities (ABTS, DPPH, FRAP) of the linseed oil samples were evaluated and summarized in Table 1. Centrifugation reduced TPC by 30 %, confirming the removal of polyphenol-rich particles, while green extraction showed a 4.4-fold increase to  $84.9 \pm 3.3$  mg GAE/kg. However, subsequent centrifugation reversed this, returning TPC to baseline levels. Interestingly, initial TPC in C-Linseed oil was higher than that reported by Fruehwirth et al. (2020) for linseed oil ( $0.27 \pm 0.11$  mg GAE/100 g seeds), but was in line with previous literature values (Zhang et al., 2011). Fruehwirth et al. (2020) attributed this to limited polyphenol transfer during cold pressing ( $\sim 0.1$  %), likely due to the hydrophilic nature of most polyphenols (Tsao, 2010). Their optimized green extraction led to TPC values of 36.5–37.1 mg GAE/kg oil, which are still considerably lower than the 84.9 mg GAE/kg we observed in GE-C-Linseed oil. This suggests that our initial polyphenol content was already higher, potentially due to differences in seed variety or storage parameters before extraction, because cold-pressing conditions remained the same.

Antioxidant assays mirrored TPC trends showing that centrifugation reduced antioxidant capacity, while green extraction significantly enhanced it. Across all three methods, Centr-Linseed oil showed significantly lower antioxidant activity than C-Linseed oil, reflecting the loss of polyphenols from press cake particles due to centrifugation. Conversely, GE-C-Linseed oil demonstrated a substantial increase in antioxidant capacity, with ABTS and DPPH values rising 5.4-fold and 5.2-fold, respectively, compared to C-Linseed oil. The FRAP assay indicated a 2.3-fold increase. The ABTS values observed in our study for conventional C-Linseed oil showed a similar radical scavenging potential as Chang et al. (2024) for flaxseed oil with  $52.9 \pm 0.7$   $\mu$ mol TE/100 g. However, the DPPH values in their study were notably higher with  $228 \pm 6$   $\mu$ mol TE/100 g. The reduction in total polyphenolic content due to filtration has the potential to negatively affect the oxidative stability

of oil over time (Fortini et al., 2016). However, the removal of moisture and suspended matter may also prevent enzymatic degradation and microbial activity, which can contribute to oxidative changes. The trade-off between oil clarity and higher nutritional quality highlights the importance of processing choices in determining the final oil composition.

### 3.1.4. Quantification of polyphenolic substances by targeted LC-MS/MS

Building on the total polyphenol content (TPC) results, targeted LC-MS/MS analyses were performed to quantify individual polyphenolic compounds in the linseed oil samples. Unlike previous studies that focused on clarified oil phases, this work specifically analyzed cloudy linseed oils enriched with suspended press cake particles following green extraction. This approach allowed for a detailed evaluation of how particle retention influences the abundance of polyphenols and which polyphenol classes are most affected by green extraction and centrifugation. The results are summarized in Fig. 2. Green extraction significantly increased total polyphenol content in linseed oil reaching  $0.58 \pm 0.07$  mmol/kg in GE-C-Linseed oil, while centrifugation consistently reduced it by removing polyphenol-rich press cake particles. Although centrifugation of green-extracted oil lowered polyphenol levels in GE-Centr-Linseed oil, they remained higher than in C-Linseed oil, highlighting the partial retention of polyphenols post-extraction. The analyzed total polyphenol concentrations were notably higher than those reported for other conventional linseed oils, with only  $0.12 \pm 0.01$  mmol/kg according to Pointner et al. (2024). This variation might be attributed to differences in seed origin, weather conditions, and cultivation practices. Our LC-MS/MS findings are consistent with the trends observed in the TPC and antioxidant capacities discussed in chapter 3.1.3. Thus, the reduction in polyphenolic substances aligned with the decreases in TPC, ABTS, DPPH, and FRAP values post-centrifugation, as confirmed by strong Pearson correlations shown in Fig. S5 ( $r \geq 0.95$ ,  $p < 0.05$ ), highlighting the direct relationship between polyphenol content and antioxidant capacity.

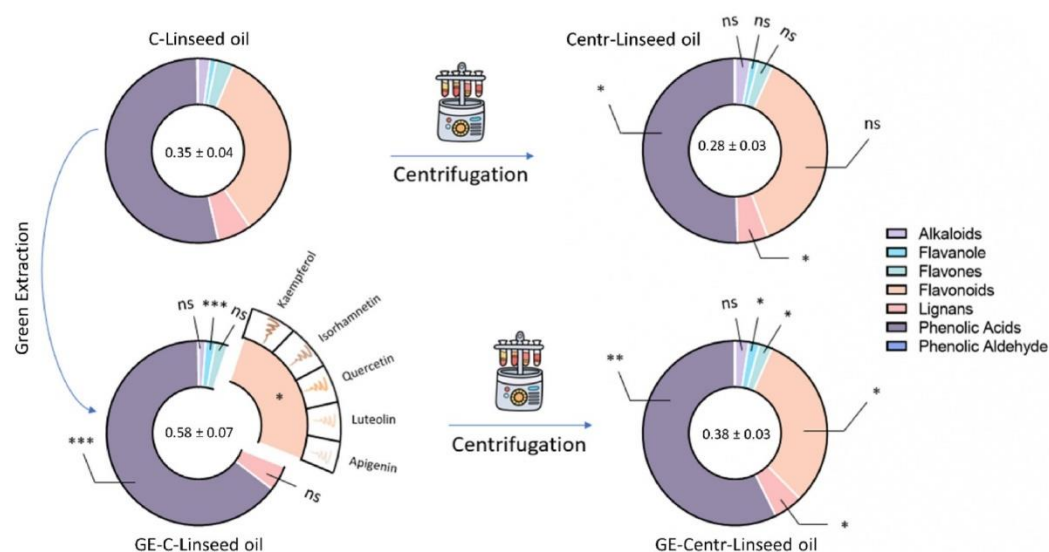
The LC-MS/MS analysis further revealed that flavonoids and phenolic acids were the most abundant polyphenol classes across samples, with their concentrations notably increasing after green extraction. This aligns with previous findings showing that flaxseed oil contains various polyphenol classes, including hydroxybenzoic acids, hydroxycinnamic acids, flavonoids and lignans (Zeb, 2021). Our results are consistent with findings by Zhang et al. (2023), who reported that flaxseed cake is a valuable source of flavonoids such as kaempferol, isorhamnetin, quercetin, luteolin and apigenin, and lignans, which contribute to enhanced antioxidant stability. Unlike Fruehwirth et al. (2020), who analyzed a few selected polyphenols in the clear oil phase, this study provided a comprehensive evaluation of polyphenol class distribution using standardized quantification with LC-MS/MS. By directly retaining press cake particles in the oil through green extraction, a holistic enrichment approach is demonstrated that preserves a broad spectrum of bioactive compounds. Additionally, these results highlight that centrifugation significantly reversed the enrichment, particularly for phenolic acids, reinforcing their strong association with insoluble press cake particles.

**Table 1**

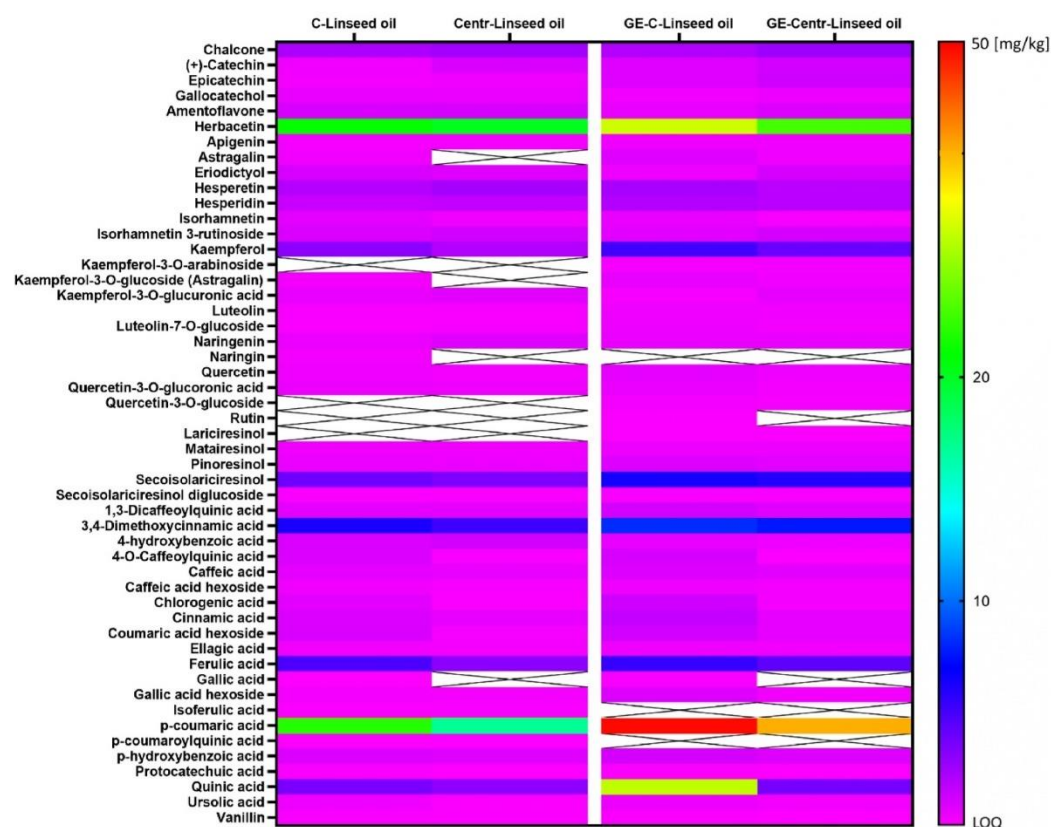
Evaluation of total polyphenol content (TPC) in mg GAE/kg and respective recovery rates in % and the antioxidant capacity values from ABTS, DPPH, both in  $\mu$ mol TE/kg and FRAP assay in  $\mu$ mol Fe(II)/kg in the analyzed linseed oil samples.

| Oil Sample           | TPC [mg GAE/kg]      | Recovery [%] | ABTS [ $\mu$ mol TE/kg] | DPPH [ $\mu$ mol TE/kg] | FRAP [ $\mu$ mol Fe(II)/kg] |
|----------------------|----------------------|--------------|-------------------------|-------------------------|-----------------------------|
| C-Linseed oil        | $19.3 \pm 1.3$       | 98.4         | $45.3 \pm 0.8$          | $25.6 \pm 1.2$          | $223.8 \pm 13.8$            |
| Centr-Linseed oil    | $13.5 \pm 1.9^*$     | 95.3         | $30.8 \pm 4.4^{**}$     | $14.3 \pm 2.5^{**}$     | $202.7 \pm 22.3^{ns}$       |
| GE-Linseed oil       | $84.9 \pm 3.3^{***}$ | 103.2        | $245.5 \pm 34.7^{****}$ | $133.8 \pm 7.8^{****}$  | $515.3 \pm 21.9^{***}$      |
| GE-Centr-Linseed oil | $18.2 \pm 0.8^{ns}$  | 101.7        | $32.2 \pm 4.7^*$        | $15 \pm 1.9^{**}$       | $235.9 \pm 11.8^{ns}$       |

One-way ANOVA, with Tukey post-hoc test was conducted, by using C-Linseed oil as control, and data are presented as mean  $\pm$  SD in mg phenolic substance/kg oil. n = 3, ns = no significance, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ , \*\*\*\* =  $p < 0.0001$ . ABTS: 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid assay, DPPH: 1,1-diphenyl-2-picrylhydrazyl assay, FRAP: ferric reducing antioxidant potential assay.



**Fig. 2.** Classification of linseed oil polyphenols into alkaloids, flavanols, flavones, flavonoids, lignans and phenolic acids as determined by LC-MS/MS. The figure shows the differences of relative profile changes from the initial C-Linseed oil sample after centrifugation and green extraction and another centrifugation step. The values in the center indicate the total polyphenol content in mmol/kg oil. Unpaired t-tests, utilizing parametric tests with a FDR approach for multiple comparisons were conducted between one and the next processing step. n = 3, ns = no significance, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .



**Fig. 3.** The heatmap shows all polyphenolic substances analyzed in the different linseed oil samples by LC-MS/MS. Red indicates high abundant mean amounts of these substances of up to 50 mg/kg oil, while purple shows low concentrations and crossed-out white fields are below limit of quantification (LOQ).



To gain deeper insights into which specific polyphenolic substances were most affected by green extraction and centrifugation, the heatmap in Fig. 3 provides a comprehensive overview across samples. 51 polyphenols were identified, with green extraction enriching the polyphenol profile. Generally, GE-C-*Linseed* oil showed the highest concentrations, while centrifugation consistently reduced polyphenol levels in both unprocessed and green-extracted oils.

The flavonoids herbacetin and kaempferol increased significantly after green extraction in C-*Linseed* oil, from  $21.09 \pm 1.11$  to  $26.63 \pm 2.53$  mg/kg ( $p < 0.05$ ) and  $3.10 \pm 0.31$  to  $5.18 \pm 0.87$  mg/kg ( $p < 0.01$ ), respectively. The results from conventional clear *linseed* oils align with Hasiewicz-Derkacz et al. (2015), who reported the presence of kaempferol and luteolin derivatives in flaxseed oil, albeit at lower concentrations. Secoisolaricresinol, the most abundant lignan, increased by 73 % after green extraction, which is similar to values reported by Fruehwirth et al. (2020), but showed better retention after centrifugation compared to other polyphenol groups, with values of  $6.04 \pm 0.65$  mg/kg in GE-Centr-*Linseed* oil. This suggests stronger oil-phase partitioning of lignans relative to flavonoids or phenolic acids, consistent with the findings of Herchi et al. (2011), who reported secoisolaricresinol, pinoresinol, and matairesinol as dominant lignans in flaxseed oil by HPLC-ESI-TOF.

Phenolic acids also increased with green extraction. p-Coumaric acid showed the highest concentrations of  $49.63 \pm 8.54$  mg/kg in GE-C-*Linseed* oil, dropping to  $38.81 \pm 7.85$  mg/kg after centrifugation, exceeding values reported by Hasiewicz-Derkacz et al. (2015) via UPLC-MS analysis. Ferulic acid followed a similar trend. Notably, 3, 4-dimethoxycinnamic acid remained stable during centrifugation in GE-Centr-*Linseed* oil, while quinic acid saw a drastic rise during green extraction in GE-C-*Linseed* oil to  $33.74 \pm 2.06$  compared to  $3.08 \pm 0.41$  in C-*Linseed* and  $3.71 \pm 0.31$  mg/kg in GE-Centr-*Linseed* oils, likely due to chlorogenic acid hydrolysis during green extraction. All quantified polyphenols are summarized in Table S3 with respective  $m/z$  precursors and fragments.

### 3.2. Lipid composition and oxidative stability

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

While polyphenols and antioxidants play a crucial role in oxidative stability, the lipid composition is an important factor influencing oil quality, degradation pathways, and functional properties. Therefore, GC-FID analyses were conducted to evaluate whether green extraction and centrifugation affect the fatty acid profiles of *linseed* oil samples. The profiles were dominated by 53 % ALA, 17 % linoleic acid, and 22 % oleic acid. Other components included stearic acid and other saturated fatty acids, which were present in smaller proportions. This distribution is common for *linseed* oils as Danish and Nizami (2019) showed. As expected, green extraction and centrifugation did not significantly alter the fatty acid profiles, as these are mainly determined by the source material and remain stable under mild processing (Shim et al., 2015).

#### 3.2.2. Hydrolysis products by $^1\text{H}$ NMR

Since the structural integrity of triacylglycerols (TAGs) is important in oil stability and quality, it is essential to assess whether green extraction and centrifugation influence the hydrolysis of TAGs into partial glycerides (DAGs, MAGs) or free glycerol (Gol).  $^1\text{H}$  NMR spectroscopy was employed to analyze the composition of the *linseed* oil samples, which is shown in Fig. S6. The graph shows the distinct concentrations of the different glycerol structures, corresponding to the hydrolysis products. TAG levels significantly decreased in cloudy oils (C-*Linseed* and GE-C-*Linseed* oils) compared to centrifuged oils ( $p < 0.0001$ ), suggesting that hydrolytic activity was more pronounced in cloudy samples due to the presence of suspended particles. DAG values were also influenced by centrifugation, particularly for 1,3-DAG, which were significantly reduced after centrifugation. C-*Linseed* oil contained

mean levels of 2.78 % 1,3-DAG, while GE-*Linseed* oil showed slightly higher values with 2.94 %, reflecting minor hydrolysis during green extraction. However, after centrifugation, 1,3-DAG dropped to 2.04 % in Centr-*Linseed* and 2.01 % in GE-Centr-*Linseed* oils ( $p < 0.01$ ), reinforcing that centrifugation limits the presence of hydrolysis products. MAGs were negligible across all samples, while Gol concentrations significantly increased in cloudy oils. These results demonstrate that centrifugation might reduce hydrolysis products, likely by removing water-associated compounds and suspended particles that may promote lipolytic activity.

#### 3.2.3. Peroxide and acid value determination

The peroxide value (PV) and acid value (AV) were measured to assess oxidative status and hydrolysis (Fig. S7–S8). C-*Linseed* and Centr-*Linseed* oils showed similar PVs and AVs of  $1.58 \pm 0.12$  and  $1.64 \pm 0.13$  mEq/kg and  $0.84 \pm 0.05$  and  $0.83 \pm 0.04$  mg KOH/g, consistent with Tsimidou et al. (2005), who found filtration had little effect on these markers. Green extraction significantly reduced PV to  $0.82 \pm 0.07$  mEq/kg ( $p < 0.001$ ), but increased AV to  $1.23 \pm 0.06$  mg KOH/g ( $p < 0.0001$ ), suggesting greater hydrolysis ongoing, likely due to enzyme activity or fatty acid release from press cake particles. Although some hydrolytic interactions were expected during green extraction, the AV remained rather low, suggesting controlled hydrolytic activity. Centrifugation partially reversed these effects, increasing PV to  $1.26 \pm 0.09$  mEq/kg ( $p < 0.05$ ) and lowering AV to  $1.01 \pm 0.05$  mg KOH/g ( $p < 0.0001$ ), similar to trends observed by Fortini et al. (2016), who noted filtered oils had higher PVs over storage due to oxygen exposure, while cloudy oils showed greater AV increases from water-facilitated lipase activity.

All values remained below Codex Alimentarius Commission (1999) limits (15 mEq  $\text{O}_2$ /kg PV, 4 mg KOH/g AV). Free fatty acids (FFA) levels, calculated from the AV, followed similar trends, rising with green extraction ( $0.59 \pm 0.03$  %) and dropping post-centrifugation ( $0.50 \pm 0.03$  %). The values of conventional clear *linseed* oils align with Rabiej-Kozioł et al. (2023) and highlight that green extraction improves the oxidative status, but promotes hydrolysis, while centrifugation balances both by removing pro-oxidative particles.

#### 3.2.4. Rancimat analysis

Building on the PV and AV results, Rancimat analysis was used to assess oxidative induction time (OIT) under accelerated conditions shown in Fig. 4. C-*Linseed* oil showed an OIT of  $2.06 \pm 0.08$  h at  $110^\circ\text{C}$ , which was slightly reduced after centrifugation. Green extraction significantly improved OIT to  $2.49 \pm 0.09$  h ( $p < 0.0001$ ), exceeding common values for *linseed* oils reported in the literature (Symoniuk et al., 2016). Post-centrifugation, GE-Centr-*Linseed* oil still maintained a high OIT of  $2.36 \pm 0.08$  h. Arrhenius-based predictions estimated a 2.78 months shelf-life at  $25^\circ\text{C}$  for C-*Linseed* oil, and reduced to 2.24 months post-centrifugation, aligning with typical flaxseed oil ranges of 5 weeks–3 months (Choo et al., 2007). Green extraction extended shelf-life by 52 % to 3.41 months, with GE-Centr-*Linseed* oil showing 2.92 months despite centrifugation. These results confirm that green extraction enhances oxidative stability, while centrifugation slightly reduced this benefit by removing polyphenol- and pigment-rich anti-oxidative particles. While the antioxidant function of chlorophyll may contribute to improved Rancimat stability, its role under real storage conditions remains complex. Light exposure may convert chlorophyll from an antioxidant into a pro-oxidant, promoting photooxidation. Future work should evaluate oil stability under both illuminated and dark storage to assess chlorophyll's net impact in realistic conditions.

#### 3.2.5. Secondary lipid oxidation products by SPME/GC-MS

Semi-quantitative SPME/GC-MS analysis evaluated secondary lipid oxidation products, focusing on aldehydes, alcohols, ketones, and furans. Volatile profiles exhibited significant changes in the analyzed oils, as shown in Fig. 5. Hexanal, a key oxidation marker, decreased by 93 %

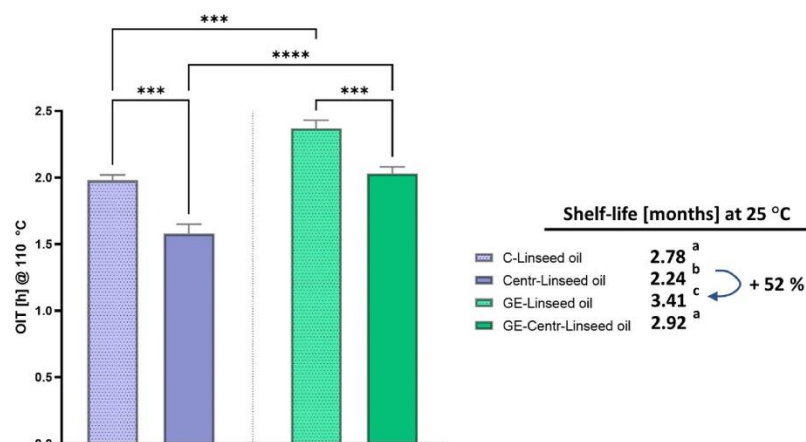


Fig. 4. The OIT [h] at 110 °C in linseed oils was analyzed by the Rancimat method and the shelf-life was predicted at room temperature (25 °C) based on the Arrhenius equation. Dotted bars indicate cloudy linseed oil samples containing press cake particles. One-way ANOVA was conducted, with Tukey post-hoc test, and data are presented as mean + SD,  $n = 3$ , \*\*\* =  $p < 0.001$ , \*\*\*\* =  $p < 0.0001$ .

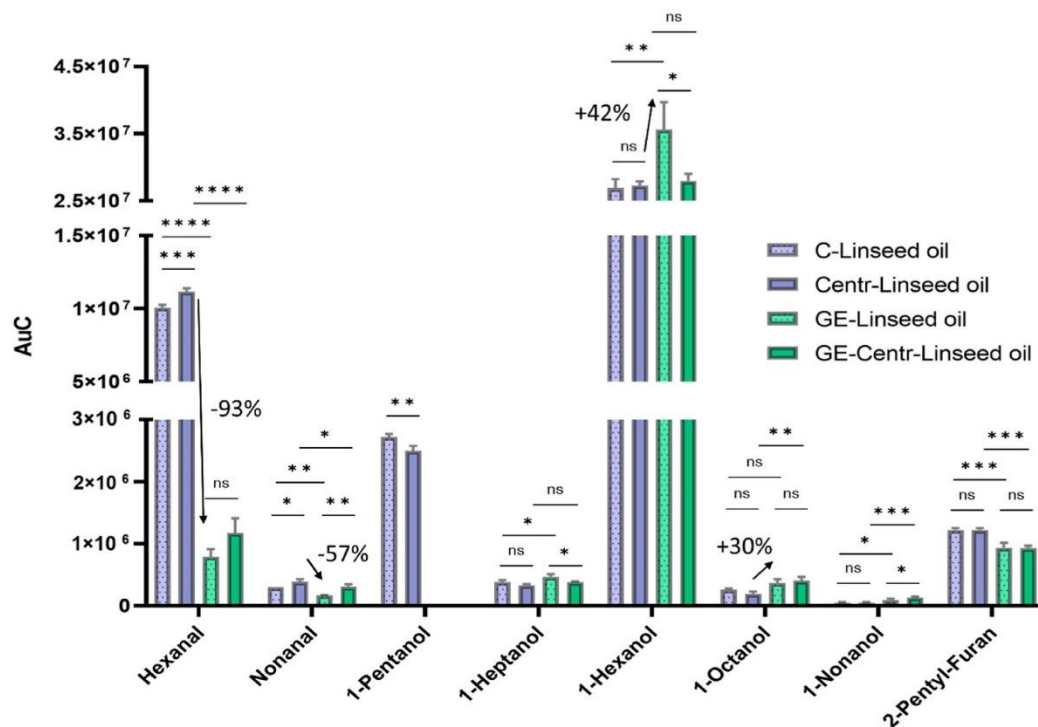


Fig. 5. Secondary lipid oxidation products detected in the analyzed linseed oil samples via GC-MS. The volatile substances are shown as area under the curve (AuC). One-way ANOVA was conducted, with Tukey post-hoc test, data are presented as mean + SD,  $n = 3$ , ns = no significance, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ , \*\*\*\* =  $p < 0.0001$ .

in GE-C-Linseed compared to C-Linseed oil, aligning with its enhanced oxidative status. Nonanal also declined by 57 %, indicating the anti-oxidative impact of green extraction, likely due to increased polyphenol and pigment content. Conversely, 1-hexanol and 1-octanol increased by 42 % and 30 %, suggesting selective formation of secondary oxidation products under green extraction conditions, while 2-pentyl-furan remained stable across samples. Krist et al. (2006) showed that volatile profiles of linseed oils vary based on extraction methods, plant

variety, and cultivation conditions, with notable differences in acetic acid, trans-2-butenal, hexanol, and 2-pentylfuran. Our results support previous studies identifying aldehydes, ketones, and alcohols as key volatiles in linseed oil oxidation (Juita et al., 2012). The significant reduction in aldehydes highlight green extraction's role in improving oxidative stability and shelf-life.

Overall, centrifuged samples consistently exhibited higher aldehyde levels compared to cloudy oils. That suggests that suspended press cake



particles contribute to aldehyde reduction, likely through interactions with antioxidants, while alcohols showed generally lower values in centrifuged samples. Similar effects were seen in native olive oils, where filtration influenced volatile profiles by removing suspended particles that carried bioactive compounds. In contrast, [Lozano-Sánchez et al. \(2010\)](#) found that non-filtered oils retained more volatile oxidation markers like aldehydes and ketones, affecting both flavor and stability. The colloidal nature of cloudy oils, with emulsified water droplets and polyphenols, impacts oxidation kinetics and aroma ([Cinelli et al., 2020](#)). In our study, volatile profile differences between cloudy and centrifuged linseed oils suggest that suspended particles affect aroma retention and oxidative stability. Future sensory evaluations should assess how these compositional changes influence taste, flavor, and aroma.

#### 4. Conclusion

This study was the first to provide an extensive characterization of the composition of cloudy cold-pressed linseed oil, a product type typically studied only in the context of olive oil. Green extraction demonstrated that the oxidative stability, polyphenol content, and antioxidant capacity can be significantly enhanced by enriching it with bioactive compounds from press cake particles. Even after centrifugation to obtain a clear oil, many of these positive effects were retained, especially maintaining an improved oxidative stability compared to conventionally processed linseed oil. The presence of press cake particles not only boosts the nutritional profile, but also offers a sustainable and solvent-free approach to valorizing an agricultural by-product, reducing waste, and contributing to a circular economy. Compared to conventional solvent-based extractions, this green method is more environmentally friendly, energy-efficient, and based on readily available plant material. Incorporating these particles enriches the oil with additional polyphenols, pigments, and flavor compounds, potentially enhancing both its functional properties and taste. Moreover, the colloidal nature of the oil with suspended bioactive particles may contribute to oxidative stabilization via cooperative interactions between particles, polyphenols, and the lipid phase. This proposed mechanism offers an interesting avenue for further investigation into natural stabilization strategies in unrefined oils. However, several limitations must be considered. First, while no harmful effects are expected based on the natural origin of the added material and the mild processing conditions, potential safety concerns related to anti-nutritional compounds, degradation products, or microbial contamination during storage should be addressed in future studies. Second, although Rancimat testing provided insight into short-term oxidative stability, no real-time storage experiments were performed under practical conditions involving light and temperature variation. Third, no sensory or consumer acceptability testing was conducted yet.

While these findings highlight the nutritional and environmental benefits of cloudy linseed oils, consumer acceptance remains a crucial factor. Attributes such as appearance, flavor, and texture may impact consumer perception, especially regarding the cloudiness.

#### 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 Auñón-Lopez:** Writing – review & editing, Methodology, Formal analysis, Data curation. **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.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2025.118034>.

#### Data availability

Data will be made available on request.

#### References

- Aktar, T., & Adal, E. (2019). Determining the arrhenius kinetics of avocado oil: Oxidative stability under rancimat test conditions. *Foods*, 8(7), 236. <https://doi.org/10.3390/foods8070236>
- Bendini, A., Valli, E., Rocculi, P., Romani, S., Cerretani, L., & Gallina Toschi, T. (2013). A new patented system to filter cloudy extra virgin olive oil. *Current Nutrition & Food Science*, 9(1), 43–51. <https://doi.org/10.2174/1573401311309010008>
- 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(1), 70–76. <https://doi.org/10.1006/abio.1996.0292>
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Chandra, S., Kumar, M., Dwivedi, P., & Shinde, L. P. (2020). Functional and nutritional health benefit of cold-pressed oils: A review. *Journal of Agriculture and Ecology*, 9(1), 21–29. <https://doi.org/10.53911/jae.2020.9102>
- Chang, K., Gao, P., Wang, S., Wei, W., Yin, J., Zhong, W., & Reaney, M. J. (2024). Tailoring oil blends for specific purposes: A study on nutritional and antioxidant properties of soybean oil mixed with corn, sunflower, and flaxseed oils. *LWT*, 206, Article 116628. <https://doi.org/10.1016/j.lwt.2024.116628>
- Choo, W. S., Birch, J., & Dufour, J. P. (2007). Physicochemical and quality characteristics of cold-pressed flaxseed oils. *Journal of Food Composition and Analysis*, 20(3–4), 202–211. <https://doi.org/10.1016/j.jfca.2006.12.002>
- Cinelli, G., Cofelice, M., & Venditti, F. (2020). Veiled extra virgin olive oils: Role of emulsion, water and antioxidants. *Colloids and Interfaces*, 4(3), 38. <https://doi.org/10.3390/colloids4030038>
- CODEX, STAN. (2011). *Codex standard for named vegetable oils*. Rome: FAO/WHO (Codex Stan 210-1999).
- Danish, M., & Nizami, M. (2019). Complete fatty acid analysis data of flaxseed oil using GC-FID method. *Data in Brief*, 23, Article 103845. <https://doi.org/10.1016/j.dib.2019.103845>
- Drévilon, L., Nemer, G., Mitri, S., El Kantar, S., Maroun, R. G., Louka, N., & Koubaa, M. (2023). Application of high-voltage electrical discharges and other alternative technologies for microbial cell lysis and extraction of biocompounds. In E. Vorobiev (Ed.), *Processing of food products and wastes with high voltage electrical discharges* (pp. 199–222). Elsevier Science & Technology. <https://doi.org/10.1016/B978-0-323-95403-7.00005-2>
- Fortini, M., Migliorini, M., Cherubini, C., Cecchi, L., Guerrini, L., Masella, P., & Parenti, A. (2016). Shelf life and quality of olive oil filtered without vertical centrifugation. *European Journal of Lipid Science and Technology*, 118(8), 1213–1222. <https://doi.org/10.1002/ejlt.201500229>
- Fruehwirth, S., Steinschaden, R., Woschitz, L., Richter, P., Schreiner, M., Hoffmann, B., Hoffmann, W., & Pignitter, M. (2020). Oil assisted extraction of polyphenols from press cake to enhance oxidative stability of flaxseed oil. *LWT*, 133, Article 110006. <https://doi.org/10.1016/j.lwt.2020.110006>
- Gliszczynska Swiglo, A., Sikorska, E., Khmelinskii, I., & Sikorski, M. (2007). Tocopherol content in edible plant oils. *Polish Journal of Food and Nutrition Sciences*, 57(4), 157–161 [A]. <https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-artic-e-c9b0ed5-1fd2-49d5-bba0-2a864a6dbbe>
- Grosshagauer, S., Steinschaden, R., & Pignitter, M. (2019). Strategies to increase the oxidative stability of cold pressed oils. *LWT*, 106, 72–77. <https://doi.org/10.1016/j.lwt.2019.02.046>



- Gülmez, Ö., & Şahin, S. (2019). Evaluation of oxidative stability in hazelnut oil treated with several antioxidants: Kinetics and thermodynamics studies. *LWT*, 111, 478–483. <https://doi.org/10.1016/j.lwt.2019.05.077>
- Haque, M., Khan, F. N., Yadav, S., Kaur, V., Kaushik, N., Kumar, A., & Langyan, S. (2024). Chemistry, bioactivity, and health effects of linseed food products as nutraceutical and dietary supplements. In *Linseed* (pp. 79–90). Elsevier. <https://doi.org/10.1016/B978-0-443-15439-3.00012-6>
- Hasiewicz-Derkacz, K., Kulma, A., Czuj, T., Prescha, A., Żuk, M., Grajzer, M., Łukaszewicz, M., & Szopa, J. (2015). Natural phenolics greatly increase flax (*Linum usitatissimum*) oil stability. *BMC Biotechnology*, 15, 62. <https://doi.org/10.1186/s12896-015-0178-0>
- 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(1), Article e057. <https://doi.org/10.3989/gya.0463141>
- Herchi, W., Sawalha, S., Arráez-Román, D., Boukhchina, S., Segura-Carretero, A., Kallel, H., & Fernández-Gutiérrez, A. (2011). Determination of phenolic and other polar compounds in flaxseed oil using liquid chromatography coupled with time-of-flight mass spectrometry. *Food Chemistry*, 126(1), 332–338. <https://doi.org/10.1016/j.foodchem.2010.10.070>
- Holler, M., Alberdi-Cedeño, J., Añón-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. <https://doi.org/10.1016/j.fpsl.2023.101051>
- ISO 660:2020. (2020). In *International standard ISO 660:2020, animal and vegetable fat and oils, determination of acid value and acidity* (4th ed.) 2020.
- Joshi, A., Hegde, M., & Zanwar, A. (2022). Flaxseed oil and palm olein blend to improve omega-6: Omega-3 ratio. *Journal of Food Science and Technology*, 59(2), 498–509. <https://doi.org/10.1007/s13197-021-05033-4>
- Juitta, Długogorski, B. Z., Kennedy, E. M., & Mackie, J. C. (2012). Identification and quantitation of volatile organic compounds from oxidation of linseed oil. *Industrial & Engineering Chemistry Research*, 51(16), 5645–5652. <https://doi.org/10.1021/ie202535d>
- Kaur, M., Singh, B., & Kaur, A. (2024). Microwave processing effects on physico-chemical, functional properties, phenolic profile, and maillard products of flaxseed flour and flaxseed press cake flour. *Industrial Crops and Products*, 218, Article 118900. <https://doi.org/10.1016/j.indcrop.2024.118900>
- Koidis, A., Triantafyllou, E., & Boskou, D. (2008). Endogenous microflora in turbid virgin olive oils and the physicochemical characteristics of these oils. *European Journal of Lipid Science and Technology*, 110(2), 164–171. <https://doi.org/10.1002/ejlt.200700055>
- Kostadinović Velickovska, S., Naumova Letia, G., Čočevska, M., Brühl, L., Silaghi-Dumitrescu, R., Mirhosseini, H., Ilieva, F., Mihajlov, L., Dimovska, V., Kovacević, B., Gulaboski, R., & Matthäus, B. (2018). Effect of bioactive compounds on antiradical and antimicrobial activity of extracts and cold-pressed edible oils from nutty fruits from Macedonia. *Journal of Food Measurement and Characterization*, 12(4), 2545–2552. <https://doi.org/10.1007/s11694-018-9871-8>
- Krist, S., Stuebiger, G., Bail, S., & Unterwieser, H. (2006). Analysis of volatile compounds and triacylglycerol composition of fatty seed oil gained from flax and false flax. *European Journal of Lipid Science and Technology*, 108(1), 48–60. <https://doi.org/10.1002/ejlt.200500267>
- Lercker, G., Frega, N., Bocci, F., & Servidio, G. (1994). “Veiled” extra-virgin olive oils: Dispersion response related to oil quality. *Journal of the American Oil Chemists’ Society*, 71(6), 657–658. <https://doi.org/10.1007/BF02540597>
- Lozano Sánchez, J., Cerretani, L., Bendini, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Filtration process of extra virgin olive oil: Effect on minor components, oxidative stability and sensorial and physicochemical characteristics. *Trends in Food Science & Technology*, 21(4), 201–211. <https://doi.org/10.1016/j.tifs.2009.12.004>
- Nieva-Echevarría, B., Goicoechea, E., Manzanos, M. J., & Guillén, M. D. (2014). A method based on 1H NMR spectral data useful to evaluate the hydrolysis level in complex lipid mixtures. *Food Research International*, 66, 379–387. <https://doi.org/10.1016/j.foodres.2014.09.031>
- Obranović, M., Škevin, D., Kraljić, K., Pospisil, M., Nederal, S., Blekić, M., & Putnik, P. (2015). Influence of climate, variety and production process on tocopherols, Plastoquinone-8 and pigments in flaxseed oil. *Food Technology and Biotechnology*, 53(4), 496–504. <https://doi.org/10.17113/ftb.53.04.15.4252>
- 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). Springer International Publishing. [https://doi.org/10.1007/978-3-030-05315-4\\_16](https://doi.org/10.1007/978-3-030-05315-4_16)
- Pignitter, M., Stolze, K., Gartner, S., Dumhart, B., Stoll, C., Steiger, G., Kraemer, K., & 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(10), 2297–2305. <https://doi.org/10.1021/jf405736j>
- Pino, L. K., Searle, B. C., Bollinger, J. G., Nunn, B., MacLean, B., & MacCoss, M. J. (2020). The skyline ecosystem: Informatics for quantitative mass spectrometry proteomics. *Mass Spectrometry Reviews*, 39(3), 229–244. <https://doi.org/10.1002/mas.21540>
- Pointner, T., Rauh, K., Añón-Lopez, A., Kostadinović Velickovska, S., Mitrev, S., Arsov, E., & Pignitter, M. (2024). Comprehensive analysis of oxidative stability and nutritional values of germinated linseed and sunflower seed oil. *Food Chemistry*, 454, Article 139790. <https://doi.org/10.1016/j.foodchem.2024.139790>
- Rabiej-Kozioł, D., Momot-Ruppert, M., Stawicka, B., & Szydłowska-Czerniak, A. (2023). Health benefits, antioxidant activity, and sensory attributes of selected cold-pressed oils. *Molecules*, 28(14). <https://doi.org/10.3390/molecules28145484>
- 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(9–10), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3)
- Shadyro, O., Sosnovskaya, A., & Edinecheva, I. (2020). Effect of biologically active substances on oxidative stability of flaxseed oil. *Journal of Food Science and Technology*, 57(1), 243–252. <https://doi.org/10.1007/s13197-019-04054-4>
- Shin, Y. Y., Gui, B., Wang, Y., & Reaney, M. J. (2015). Flaxseed (*Linum usitatissimum* L.) oil processing and selected products. *Trends in Food Science & Technology*, 43(2), 162–177. <https://doi.org/10.1016/j.tifs.2015.03.001>
- 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. In *Methods in enzymology. Oxidants and antioxidants part A* (Vol. 299, pp. 152–178). Elsevier. [https://doi.org/10.1016/S0076-6879\(99\)90017-1](https://doi.org/10.1016/S0076-6879(99)90017-1)
- Sun, X., Wan, Y., Han, J., Liu, W., & Wei, C. (2023). Analysis of volatile compounds and flavor fingerprint in hot-pressed flaxseed oil processed under different roasting conditions using headspace gas chromatography-ion mobility spectrometry. *Food Analytical Methods*, 16(5), 888–899. <https://doi.org/10.1007/s12161-023-02467-8>
- Symoniuk, E., Ratusz, K., & Krygier, K. (2016). Comparison of the oxidative stability of linseed (*Linum usitatissimum* L.) oil by pressure differential scanning calorimetry and Rancimat measurements. *Journal of Food Science and Technology*, 53(11), 3986–3995. <https://doi.org/10.1007/s13197-016-2398-2>
- Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients*, 2(12), 1231–1246. <https://doi.org/10.3390/nu2121231>
- Tsimidou, M. Z., Georgiou, A., Koidis, A., & Boskou, D. (2005). Loss of stability of “veiled” (cloudy) virgin olive oils in storage. *Food Chemistry*, 93(3), 377–383. <https://doi.org/10.1016/j.foodchem.2004.09.033>
- Wheeler, D. H. (1932). Peroxide formation as a measure of autooxidative deterioration. *Journal of the American Oil Chemists’ Society*, 9(4), 89–97. <https://doi.org/10.1007/BF02553782>
- Zeb, A. (2021). A comprehensive review on different classes of polyphenolic compounds present in edible oils. *Food Research International*, 143, Article 110312. <https://doi.org/10.1016/j.foodres.2021.110312>
- Zhang, Z.-S., Wang, L.-J., Li, D., Li, S.-J., & Özkan, N. (2011). Characteristics of flaxseed oil from two different flax plants. *International Journal of Food Properties*, 14(6), 1286–1296. <https://doi.org/10.1080/10942911003650296>
- Zhang, T., Zhang, Z., & Wang, X. (2023). Composition and antioxidant ability of extract from different flaxseed cakes and its application in flaxseed oil. *Journal of Oleo Science*, 72(1), 59–67. <https://doi.org/10.5650/jos.ess22181>

## 4 PART II: PACKAGING AND FORTIFICATION STRATEGIES

### 4.1 RESEARCH SCOPE

Part II of this thesis explored approaches to preserve the quality and oxidative stability of edible oils, focusing on the role of formulation and packaging material in mitigating lipid oxidation during storage. While the preceding part addressed antioxidant enrichment more at the production level, this part concentrated on interventions, through either fortification of oils with natural stabilizing agents or the use of bioplastic packaging materials. Together, these studies aimed to clarify how both the oil composition and its packaging material determine shelf-life and consumer safety.

The first study investigated the fortification of polyunsaturated, cold-pressed vegetable oils, namely, walnut, safflower and linseed oil, with the aminophospholipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (PE(16:0/18:1)). Aminophospholipids are natural constituents of biological membranes known to exert antioxidative effects via multiple mechanisms, including LOOH decomposition, free-radical scavenging and carbonyl trapping. The study aimed to determine whether supplementation with PE(16:0/18:1) could enhance oxidative stability under both accelerated and household storage conditions, and whether its protective effects were temperature-dependent. The evaluation combined Rancimat analysis,  $^1\text{H}$  NMR and SPME-GC-MS to track primary and secondary oxidation markers, as well as tocopherol dynamics. Mechanistic assays further explored its potential to trap lipid-derived aldehydes. Collectively, the project provided mechanistic insight into phosphatidylethanolamine as a multifunctional natural antioxidant, offering a biologically inspired strategy to stabilize PUFA-rich oils without synthetic additives.

The second work addressed the impact of packaging materials on lipid oxidation, comparing conventional plastics (PET, r-PET, PE, bio-PE) with the biodegradable alternative polylactic acid (PLA) during sunflower oil storage. This work assessed how different packaging material influence oxidation kinetics and tocopherol depletion under household-relevant conditions. Using complementary analytical methods (peroxide value,  $^1\text{H}$  NMR, SPME-GC-MS, and LC-MS/MS), the study also monitored potential migration of packaging-

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derived compounds, notably benzene and PLA-related oligomers, to evaluate food safety aspects. The results demonstrated that PLA provided oxidative protection comparable to PET up to two months of storage, without detectable NIAS, highlighting its potential as a renewable and safe packaging alternative for edible oils.

Overall, Part II bridges formulation and packaging aspects, illustrating how the internal composition of an oil and its external packaging material contribute to maintaining quality and preventing oxidation. By integrating phospholipid-based fortification with sustainable packaging evaluation, this part provides a comprehensive perspective on extending oil shelf-life and reducing environmental impact through naturally derived and biodegradable solutions.

## 4.2 Original Works

### 4.2.1 Polylactic acid as a promising sustainable plastic packaging for edible oils

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### **Highlights**

- Packaging material affects sunflower oil oxidative stability during storage.
- Sunflower oil in PE and Green-PE bottles oxidizes faster than in PET or PLA bottles.
- Oxidative stability of oils in PLA was similar to PET and r-PET within two months.
- Benzene was detected in PET samples, no NIAS were observed in PLA samples.
- PLA seems to be an alternative packaging material for edible oils replacing PET.



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## Polylactic acid as a promising sustainable plastic packaging for edible oils

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## ABSTRACT

The influence of renewable packaging materials on the oxidative stability of sunflower oil was investigated to evaluate whether they could be used as alternatives to conventional plastics. Two renewable bottle materials, polylactic acid (PLA) and bio-polyethylene (Green-PE) were compared to conventional plastics consisting of virgin and recycled polyethylene terephthalate (PET, r-PET) and regular polyethylene (PE), in a storage study over a period of 56 days. The results showed that the progress of lipid oxidation in PLA was similar to PET and r-PET until day 28, while it was significantly increased in PE and Green-PE. Benzene was detected as the only migration compound in the oil stored in PET and r-PET, with concentrations of  $0.153 \pm 0.027 \mu\text{g/g}$  and  $0.187 \pm 0.024 \mu\text{g/g}$  after 56 days of storage. The study concluded that PLA could be used as an alternative packaging material for edible oils to replace PET.

## 1. Introduction

Vegetable oils are one of the most prevalent food products in the human diet and increasing attention is being paid to them due to their importance in the food industry, in nutrition and in human health. Vegetable oils are a source of essential fatty acids as well as of some other minor compounds, such as vitamins, sterols, polyphenols and squalene, to which positive biological effects have been attributed (Gunstone, 2011; Alberdi-Cedeño et al., 2017). Unfortunately, both main and minor compounds naturally present in the oils are susceptible to oxidation reactions which result in a deterioration of their quality and in the formation of a plethora of oxidized compounds (Grüneis et al., 2019; Alberdi-Cedeño et al., 2019). Due to the oxidation of the acyl groups, hydroperoxides are formed, further breaking into secondary and tertiary oxidation compounds (Frankel, 2005; Laguerre et al., 2020; Schaich, 2012). Oxidation is a very complex sequence of transformations. It should be noted that, although there are long-accepted general mechanisms for oil oxidation processes (the classical three stages: initiation, propagation and termination) (Frankel, 2005), the mechanism by which these processes occur is still not fully understood.

Moreover, alternative pathways by which the oxidation processes take place have been also described being very different from one case to another, depending on many factors (Schaich, 2012). Thus, the rate of oxidation is influenced by many factors such as the degree of lipid unsaturation, the type and the concentration of oxygen, the temperature as well as light irradiation and the presence and the concentration of minor compounds or molecular species exhibiting either antioxidant or prooxidant activities (Choe & Min, 2006; Pignitter et al., 2014; Martínez-Yusta et al., 2014; Grosshagauer et al., 2019).

Besides these factors, the packaging of vegetable oils is of decisive importance and can affect the final quality of them. Therefore, the choice of the packaging material for such sensitive products is crucial to protect them from lipid oxidation, unwanted biological, mechanical, or physical alterations as well as to prevent contamination of the oil (Shaikh et al., 2021). Several materials, such as glass, metal, and different kinds of plastic films, might be used for packaging. Depending on their O<sub>2</sub> permeability and light transmittance the oxidative stability of the oils and therefore their shelf life and quality may be directly affected (Huyan et al., 2019). Some oils, like extra virgin olive or linseed oils are stored in glass bottles. However, other vegetable oils, such as

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sunflower oil, are stored in transparent plastic containers made of polyethylene terephthalate (PET). Besides PET having good mechanical properties and a low gas permeability to protect the edible oils from being exposed to oxygen, it is also a much lighter material in comparison to glass (Nisticò, 2020). Upon usage PET bottles mostly generate a lot of waste. In 2018, 359 million tons of plastic were produced worldwide, but in Europe only about a third of it was recycled (European Parliament, 2018). Net demand for PET in the EU27 +UK was estimated at 5.1 Mt in 2020, of which 3.0 Mt was supplied from virgin PET production, 1.3 Mt from r-PET production. PET degrades very slowly in nature and can remain for up to 2000 years (Naser et al., 2021). Due to this concern, alternative packaging materials are still being explored. Some alternative packaging materials that can be obtained from renewable food sources, such as corn, potatoes, or sugar cane, are bio-polyethylene (Green-PE) and polylactic acid (PLA) (Naser et al., 2021).

These bioplastics show similar mechanical properties as PET while being more sustainable for the environment. However, they are currently more expensive and are facing several hurdles before they can be considered as a green alternative. One limitation is the need of specific composting utilities for each bioplastic and the lack of research about the migration plastic compounds into food matrices (Rahman & Bhoi, 2021). In the case of PLA, the most promising material from the above-mentioned, lactic acid, lactoylactic acid and other small oligomers for polylactides have been confirmed as likely migration compounds into food simulants (Ubeda et al., 2019). Polymeric migration has also been confirmed for PE, although the structural identities of compounds that take part of it are not fully elucidated (Katsara et al., 2021). However, it is important to ensure the migration of compounds from these alternative packaging materials remains below the overall migration limit (OML) of 60 mg/kg food (European Union, 2011).

Some studies have investigated the differences in the shelf life and quality of edible oils stored in a variety of plastic packaging materials (Sharma et al., 1990; Tawfik & Huyghebaert, 1999; Ramezani, 2007; Hu et al., 2020). These studies have confirmed high-quality properties of materials such as PET and PE, in regard to parameters such as formation of hydroperoxide in the oils. However, information on other oil oxidation parameters is still unexplored, especially in the case of PLA. Furthermore, these studies applied either accelerated aging conditions, like high temperature, and only studied the effect of conventional plastic packaging materials or films as packaging materials. So far, no data is available regarding the comparison of the effect of conventional plastic materials and renewable and/or biodegradable plastic packaging on the oxidative stability of vegetable oils. It is also relevant to point out that, even though alternative materials such as PLA have been reported to possess a higher oxygen permeability compared to conventional packaging materials such as PET, other properties like thickness, color and light transmittance may impact the progress of the oxidation process in oils, which evidences the need of analyzing the performance of these materials in this regard (Naser et al., 2021).

The aim of the current study was to investigate and compare the effect of the plastic packaging materials PE, PET, recycled PET (r-PET), Green-PE and PLA on the oxidative stability and shelf life of sunflower oil stored at retail and household conditions under cold fluorescent light. Attention was paid to the packaging-induced effects on major and some minor lipid compounds (tocopherols), as well as to the evolution of the lipid oxidation products. Moreover, compounds that migrated from the bottle materials to the oils were analyzed.

## 2. Materials and methods

### 2.1. Samples

The study was carried out with sunflower oil acquired from a local supermarket (Vienna, Austria). All the sunflower oil belonged to the same batch. Its composition in acyl groups was determined from  $^1\text{H}$  NMR spectral data as in previous studies (Guillén & Uriarte, 2012),

showing values of  $55.45 \pm 0.16\%$ ,  $32.47 \pm 0.18\%$  and  $12.06 \pm 0.27\%$  for linoleic (L), oleic or monounsaturated (O or MU) and saturated (S) acyl groups, respectively. Its composition in tocopherols, determined by High Performance Liquid Chromatography (HPLC) as in previous studies (Fruehwirth et al., 2020), was:  $471.4 \pm 7.5$  mg/kg of  $\alpha$ -tocopherol and  $76.2 \pm 2.0$  of mg/kg  $\gamma$ -tocopherol. The sunflower oil was packaged in plastic bottles with a capacity of 250 mL purchased from different manufacturers and made of different plastic materials: polylactic acid (PLA) (NaKu e.U., Wiener Neustadt, Austria), recycled polyethylene terephthalate (r-PET) (Flakado, Mannheim, Germany), polyethylene terephthalate (PET) (Plasticflessen.nl, Zuidbroek, Netherlands), polyethylene (PE) and bio-polyethylene (Green-PE) (H. H. Rotert GmbH & Co. KG, Bad Idburg, Germany). PLA bottles had a thickness of  $0.7 \pm 0.05$  mm and showed clear slight-yellow colors, whereas PET and r-PET bottles were thinner ( $0.3 \pm 0.05$  mm) and clear, with no color. PE and Green-PE bottles had the highest thickness ( $1.0 \pm 0.05$  mm) and showed a white milky color.

### 2.2. Chemicals

Methanol (LC-MS Grade), water (LC-MS Grade), acetonitrile (LC-MS Grade), formic acid and isooctane were purchased from Avantor/VWR International Inc. (Radnor, PA, USA). Chloroform, acetic acid, and 0.1 M sodium thiosulfate were obtained from Carl Roth GmbH & CO. (Karlsruhe, Germany) and deuterated chloroform was bought from Eurisotop (Andover, USA). Hexane, 2-propanol, starch, tetrahydrofuran, *p*-anisidine, benzene,  $\alpha$ -tocopherol, and  $\gamma$ -tocopherol were acquired from Sigma Aldrich (St. Louis, MO, USA). Rac-Tocol was obtained from Abcam (Cambridge, United Kingdom).

### 2.3. Study design

About  $225.5 \pm 0.1$  mL of sunflower oil were filled into the plastic bottles (PET, r-PET, PE, Green-PE, and PLA), which ensured that the headspace was the same for all bottles. The bottles were closed with a cap and were stored at room temperature at equal distances from each other in a cabinet, with light conditions as described in a previous study (Pignitter et al., 2014). They were exposed to cold fluorescent light for 12 h per 24 h. Samples were taken after 1, 3, 7, 14, 28 and 56 days, and stored at  $-80^\circ\text{C}$  until analysis. The experiments were carried out in quadruplicates, which means that sampling of four bottles per material was performed per day. The fresh sunflower oil was analyzed as a control sample.

### 2.4. Study of the oxidative stability of oil during storage

#### 2.4.1. Proton nuclear magnetic resonance ( $^1\text{H}$ NMR)

$^1\text{H}$  NMR spectra of the samples were acquired using a Bruker Avance 400 spectrometer operating at 400 MHz. Briefly, 200  $\mu\text{L}$  sample were added to 400  $\mu\text{L}$  deuterated chloroform which contained 0.2% non-deuterated chloroform and a small amount (0.03%) of tetramethylsilane as internal reference. The following acquisition parameters were used as in previous studies (Martínez-Yusta & Guillén, 2014): spectral width 6410 Hz, relaxation delay 3 s, number of scans 64, acquisition time 4.8190 s, and pulse width  $90^\circ$ , with a total acquisition time of 8 min 38 s. The identification and quantification of linoleic acyl groups, hydroperoxy-*Z,E*-conjugated dienes and total hydroperoxides, were performed as reported in previous works (Guillén & Ruiz, 2003; Guillén & Ruiz, 2005; Guillén & Uriarte, 2012; Martínez-Yusta & Guillén, 2014).

To this aim, the concentration of linoleic acyl groups (%L), expressed as molar percentage, was estimated by means of the following equation:

$$\%L = 100(2A_A/3A_B)$$

In this equation,  $A_A$  is the area of the signal of *bis*-allylic protons of linoleic acyl groups and  $A_B$  is the area of the signals due to the glycerol protons at the *sn*-1 and *sn*-3 positions of triglyceride (TG) (see signal B in



Fig. S1).

The concentrations of hydroperoxy-*Z,E*-conjugated dienes and total hydroperoxides, throughout the storage time, were determined by means of the following equations:

$$[\text{Hydroperoxy} - Z, E - \text{conjugated dienes}] = [(A_a)/(A_B/4)] * 1000$$

$$[\text{Total Hydroperoxides}] = [(A_b)/(A_B/4)] * 1000$$

In these equations,  $A_a$  is the area of signal **a** corresponding to one of the protons of hydroperoxy-*Z,E*-conjugated dienic systems,  $A_b$  the area of the signal **b** corresponding to the proton of the hydroperoxide groups and  $A_B$  the area of the protons at *sn*-1 and *sn*-3 positions in the glycerol backbone of TG (see signal **B** in Fig. S1). The concentrations thus determined are given in millimoles per mol of triglyceride (mmol/mol TG).

#### 2.4.2. Peroxide value

The peroxide value (POV) was determined by the method according to Wheeler (1932) with slight modifications. A total of 5 g oil was mixed with 20 mL acetic acid/chloroform solution (3:2 v/v), and 200  $\mu$ L saturated potassium iodide solution were subsequently added. After shaking for one minute, 12 mL distilled water were added, and the sample was titrated with a 0.1 N sodium thiosulfate solution. The POV was calculated as followed:

$$\text{POV meqO}_2 / \text{kg oil} = \frac{V * N * 1000}{m}$$

V ... volume titrated (mL).

N ... normality of sodium thiosulfate.

m ... weight of sample (g).

#### 2.4.3. Solid phase microextraction followed by gas chromatography/mass spectrometry (SPME-GC/MS)

The analysis of the volatile components was done as described in a previous study (Guillén et al., 2005). For this purpose, 2.5 g oil was transferred to a 20 mL glass vial and a fiber coated with DVB/CAR/PDMS (divinylbenzene/carboxen/ polydimethylsiloxane (50/30  $\mu$ m film thickness, 1 cm length)) was used to extract the volatile compounds. A pre-equilibrium of 5 min was set before the fiber was injected into the headspace of the vial at 50 °C for 55 min. It was then inserted into the injection port and remained there for 10 min for desorption of the extracted components. The injection mode was splitless with a 5 min purge time. The GC/MS (GC-QP2010 Ultra, of Shimadzu, Korneuburg, Austria) was equipped with a Zebron ZB-5 ms column (30 m x 0.25 mm x 0.25  $\mu$ m Phenomenex, Aschaffenburg, Germany), and helium as a carrier gas with a constant pressure at 117 kPa. The temperatures of the injector and interface were set at 250 °C and 305 °C, respectively. The initial temperature of the oven was kept at 50 °C for 5 min, increased to 300 °C at a rate of 4 °C per minute and was kept there for another 30 min. Ion source and quadrupole mass analyzer temperatures were held at 230 °C and 150 °C respectively. An ionization energy of 70 eV was used. The compounds were identified by matching of their mass spectra with spectra from a commercial library by more than 85% (NIST, ver. 11.0 library). Semi-quantification was based on arbitrary units of the base peak ion area counts. The purpose of this study was not the determination of absolute but of relative concentrations that are valid for comparative purposes. All the determinations were carried out in quadruplicate in order to obtain a mean value with the corresponding standard deviation for each of the components studied.

#### 2.4.4. *p*-anisidine value

*p*-anisidine value (*p*-aV) was measured according to the method of AOCs (Cd 18–90) with slight modifications. The *p*-anisidine reagent was prepared prior to the measurement, with 25 mg *p*-anisidine being added to 10 mL glacial acetic acid. A total of 1.5 g of sample were dissolved in

25 mL isooctane. To 5 mL of the sample solution 1 mL *p*-anisidine reagent was added and subsequently incubated in the dark for 10 min. The samples were transferred into a 96-well plate and measured at an absorbance of 350 nm with a Spark photometer (Tecan Group Ltd., Switzerland) before (*Ab*) and after *p*-anisidine was added (*Aa*). The value was calculated with the sample weight in grams (*m*) as followed:

$$p - aV = \frac{25 * (1.2Aa - Ab)}{m}$$

*p*-anisidine values of 0.2 and 1.8 were obtained as the limit of detection (LOD) and limit of quantification (LOQ), respectively.

#### 2.4.5. Quantification of tocopherols by high performance liquid chromatography (HPLC)

The tocopherol content was determined according to Fruehwirth et al. (2020). Briefly, 50 mg oil were mixed with 1 mL 2-propanol and filtered through a 0.2  $\mu$ m nylon syringe filter (Phenomenex, Phenex™, NY 15 mm, 0.2  $\mu$ m). A total of 1  $\mu$ L rac-tocol (5  $\mu$ g/mL) was added to the samples and used as an internal reference. The samples were analyzed by the LC-20 (Shimadzu, Korneuburg, Austria) equipped with a C18 column (Shim-pack VP-ODS 5  $\mu$ m, 150 mm x 4.6 mm, Shimadzu, Korneuburg, Austria) and a photodiode array detector (SPD-M30A, Shimadzu, Korneuburg, Austria) at 10 °C. The flow rate was 0.5 mL/min. The mobile phase was water (solvent A) and methanol (solvent B). The gradient was 0–4 min 95% methanol, 4–36 min 100% methanol, 36–38 min 95% methanol. The tocopherols were measured at a wavelength of 294 nm and were quantified using a standard curve, with a linear concentration ranging from 19.5 to 625.0 mg/kg for both  $\alpha$ -tocopherol and  $\gamma$ -tocopherol. A limit of quantification (LOQ) of 70.0 mg/kg and 26.5 mg/kg for  $\alpha$ -tocopherol and  $\gamma$ -tocopherol, respectively, was determined by the signal to noise ratio of 10. The recovery of tocol in the samples was 90  $\pm$  10%.

#### 2.5. Analysis of migrated compounds from the bottle to the contained oil

##### 2.5.1. Liquid chromatography/mass spectrometry (LC-MS)

As PLA is a novel and promising packaging material, this study determined whether migration products and potential PLA derived compounds can migrate into the sunflower oil under the specific storage conditions. For this purpose, these compounds were analyzed in both, the bottle material and in the oil on day 1 and 56. For the bottle material, the extraction of the compounds was made based on the method previously described by Baumjohann and Harms (2015), and the preparation was done according to Tsochatzis et al. (2022) with some modifications. A total of 40 mg of the bottle was cut out around the middle area, dissolved in 2 mL tetrahydrofuran (THF), and stored in a drying oven at 55 °C for 2 h. Subsequently, the solution was evaporated under a stream of nitrogen and the residue was reconstituted in 600  $\mu$ L of acetonitrile. It was then filtered through a 0.45  $\mu$ m PVDF syringe filter. Samples were separated on a C18 column (Atlantis T3 3  $\mu$ m 2.1 mm x 150 mm, Waters) connected to a Dionex Ultimate 3000 (Thermo Fisher Scientific, Vienna, Austria) system at 25 °C. The analytes were detected by a microToF-QII (Bruker Corporation, Billerica, MA, USA). A mobile phase consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was selected, with the following gradient: 0–6 min 40% acetonitrile, 6–20 min 75% acetonitrile, 20–32 min 100% acetonitrile, 32–50 min 40% acetonitrile at a flow rate of 0.1 mL/min. MS<sup>1</sup> measurement took place in positive scan mode (ESI<sup>+</sup>) with a mass range of *m/z* 100–1000. The following ESI ion source settings were applied: capillary voltage: of 3.2 kV; nebulizer pressure of 1.2 bar (N<sub>2</sub>), a dry gas flow of 8.0 L/min (N<sub>2</sub>) and dry temperature of 180 °C. Data-dependent MS<sup>2</sup> analysis were performed using a collision energy of 35 V in product ion scan mode, with a mass range of *m/z* 100–1000. For measurement of PLA-derived migration compounds that might have transferred into the oil stored in the PLA bottles for 1



and 56 days, 100  $\mu$ L oil were diluted in 600  $\mu$ L acetonitrile, vortexed and then filtered using a 0.45  $\mu$ m PVDF syringe filter. The measurement was done with the same chromatographic method and MS settings. Identification of the migration compounds was done by comparing the MS spectra obtained to those previously described by Ubeda et al. (2019).

### 2.5.2. Quantification of benzene by SPME-GC/MS

To confirm and quantify benzene in the samples stored in different plastic materials, a standard curve spiking benzene at different concentrations in sunflower oil was acquired through SPME-GC/MS, applying the same GC settings as described above (Section 2.4.3.). A linear concentration ranging from 0.003 to 0.595  $\mu$ g/g was used for the quantification of benzene in all samples. A limit of detection (LOD) of  $0.004 \times 10^{-1}$   $\mu$ g/g and a LOQ of  $0.011 \times 10^{-1}$   $\mu$ g/g were calculated by a signal to noise ratio of 3.3 and 10, respectively.

### 2.6. Statistical analysis

Statistical analysis was performed with GraphPad Prism 9.4.1. A Shapiro-Wilk test was performed to confirm normal distribution followed by a one-way analysis of variance (ANOVA) for benzene and for the other compounds (Figs. 1–4) a two-way ANOVA followed by Tukey's post hoc test. Results were considered to be significant with a *p*-value lower than 0.05. For each experiment (plastic material) and for each day of sampling, four independent samples (different bottles) were measured.

## 3. Results and discussion

The purpose of the current study was to study the oxidative stability of sunflower oil stored in PET, r-PET, PE, Green-PE, and PLA bottles under retail and household conditions, and investigate whether the biodegradable and renewable plastics could be a good alternative to the conventional ones for storing edible oils.

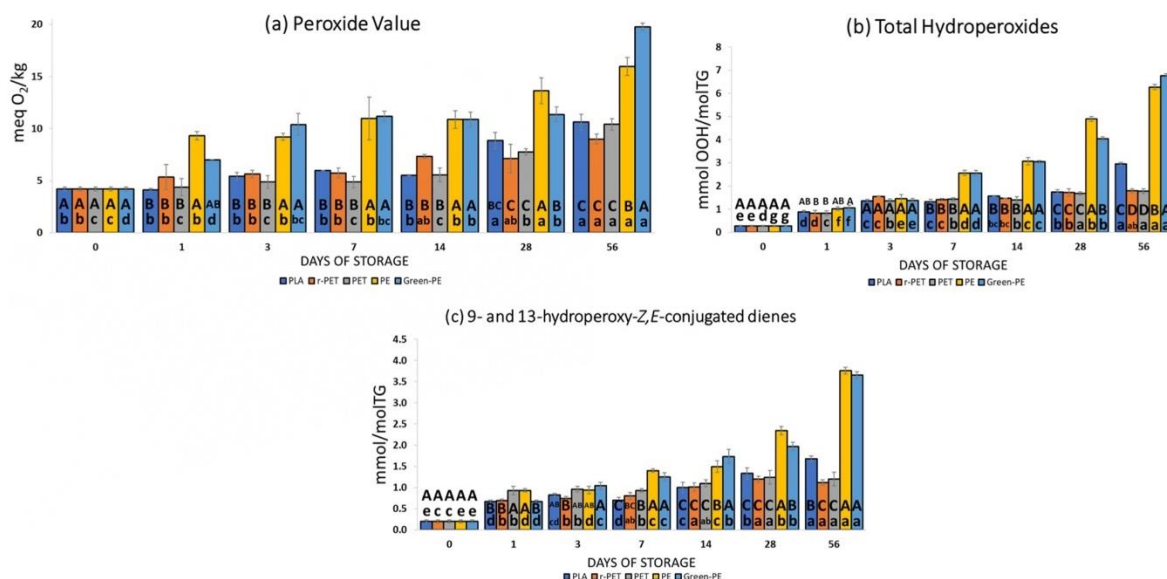
### 3.1. Impact of plastic materials on the linoleic acyl groups during storage

As mentioned before, the sunflower oil used in this study mainly consists of linoleic acyl groups ( $55.5 \pm 0.2\%$ ).  $^1\text{H}$  NMR of sunflower oil samples stored in different bottles revealed that the concentration of linoleic acyl groups remained stable over the time with no significant differences between the packaging materials (Table 1). The overall extent of lipid oxidation in the current study seemed to be low as the determination of the molar percentage of linoleic groups did not reveal any significant changes over storage time and packaging material. In this sense, Ha et al. (2011) analyzed the change of fatty acid composition of corn oil by headspace GC-MS after conducting a 2-month storage study at room temperature. Corn oil has a similar composition to sunflower oil, with linoleic and oleic as the main acyl groups. These authors did not observe any change in the concentration of linoleic acyl groups, despite an increase in lipid oxidation measured by peroxide value and the content of hexanal.

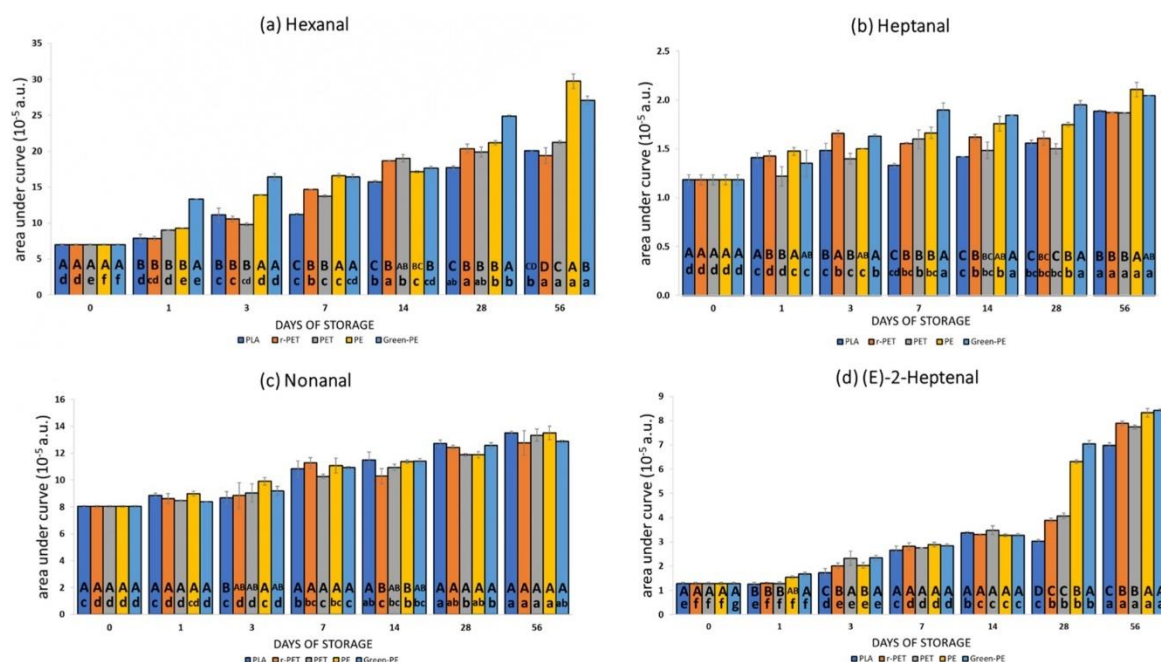
### 3.2. Occurrence of primary oxidation compounds

In addition to the aforementioned linoleic acyl groups, other more sensitive oxidation markers are the hydroperoxides. They are the primary oxidation compounds derived directly from the oxidation of unsaturated acyl groups.

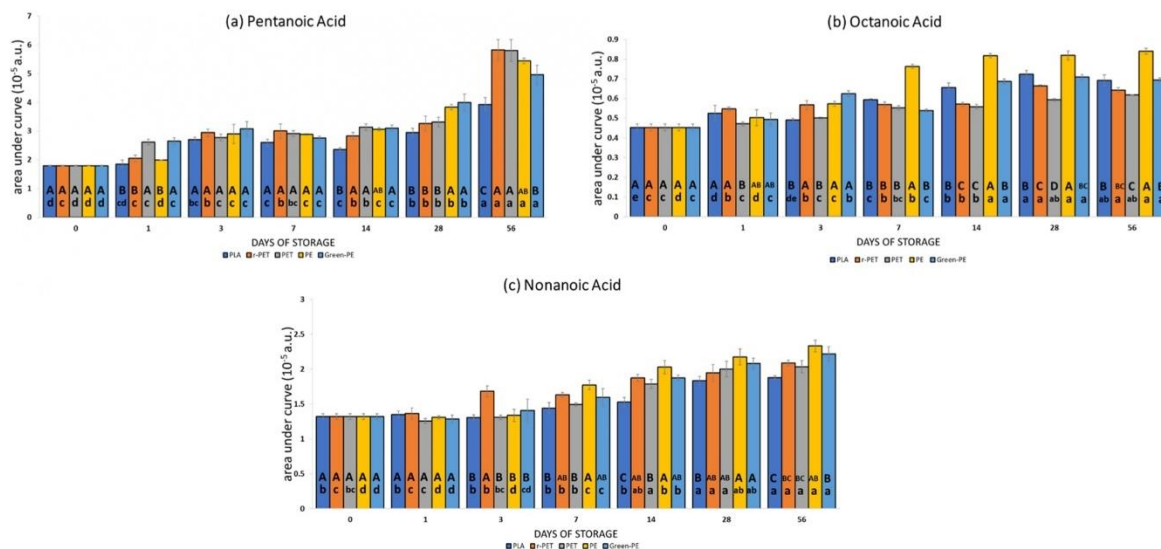
To evaluate the occurrence of primary oxidation compounds in differently packaged sunflower oil the peroxide value (POV) was determined (Fig. 1a). It is evident that the fresh sunflower oil at day 0 was slightly oxidized but was still below recommended threshold of 10 meq  $\text{O}_2/\text{kg}$  (Codex Alimentarius Commission, 1999). During the storage for 56 days, the POV increased significantly in all samples, although some differences were observed depending on the packaging material ( $10.6 \pm 0.8$ ,  $9.0 \pm 0.5$ ,  $10.4 \pm 0.6$ ,  $16.0 \pm 0.9$  and  $19.8 \pm 0.4$  meq  $\text{O}_2/\text{kg}$  for PLA, r-PET, PET, PE and Green-PE, respectively) (Fig. 1a). This could be related to the plastic bottle characteristics, wall thickness and color, leading to a higher oxidation the lower the thickness and the higher the transparency. However, looking at the bottles'



**Fig. 1.** Progress of lipid peroxidation during storage of sunflower oil in different packaging materials. Evolution of (a) peroxide value, expressed as meq  $\text{O}_2/\text{kg}$ , and the concentration, expressed as mmol/mol TG, of (b) total hydroperoxides and (c) 9 and 13-hydroperoxy-Z,E-conjugated dienes. Different capital letters show significant differences between the plastic material for each day. Different lowercase letters show significant differences for each material over the time of 56 days. Data are shown as mean  $\pm$  SD.



**Fig. 2.** Evolution of (a) hexanal, (b) heptanal, (c) nonanal and (d) (E)-2-heptenal in sunflower oil during storage in different packaging material expressed as area counts of their mass spectra base peak. Different capital letters show significant differences between the plastic material for each day. Different lowercase letters show significant differences for each material over the time of 56 days. Data are shown as mean  $\pm$  SD.



**Fig. 3.** Evolution of (a) pentanoic acid, (b) octanoic acid and (c) nonanoic acid in oil during storage in different packaging material expressed as area counts of their mass spectra base peak. Different capital letters show significant differences between the plastic material for each day. Different lowercase letters show significant differences for each material over the time of 56 days. Data are shown as mean  $\pm$  SD.

characteristics mentioned above and the obtained results the opposite was observed. Therefore, it can be assumed that the lower oxidative stability of samples stored in PE bottles could be related to the oxygen permeability of the plastic material. The oxygen permeability of PLA lies between 6.2 and 17.3  $\text{cm}^3 \text{mm}/(\text{m}^2 \text{day bar})$  depending on the ratio between L-lactic acid and D-lactic acid, but it is still higher than for

r-PET and PET at 1.2 – 2.8  $\text{cm}^3 \text{mm}/(\text{m}^2 \text{day bar})$ , while the permeability of PE is estimated to be at 202.7  $\text{cm}^3 \text{mm}/(\text{m}^2 \text{day bar})$  (Lagaron et al., 2005; Bao et al., 2006; Guinault et al., 2010; Nisticò, 2020). However, the oxygen permeability of PLA was determined with PLA as a film material of  $\mu\text{m}$  scaling. Thus, the barrier properties as a bottle with thicker material should be better than the ones from the film. Although

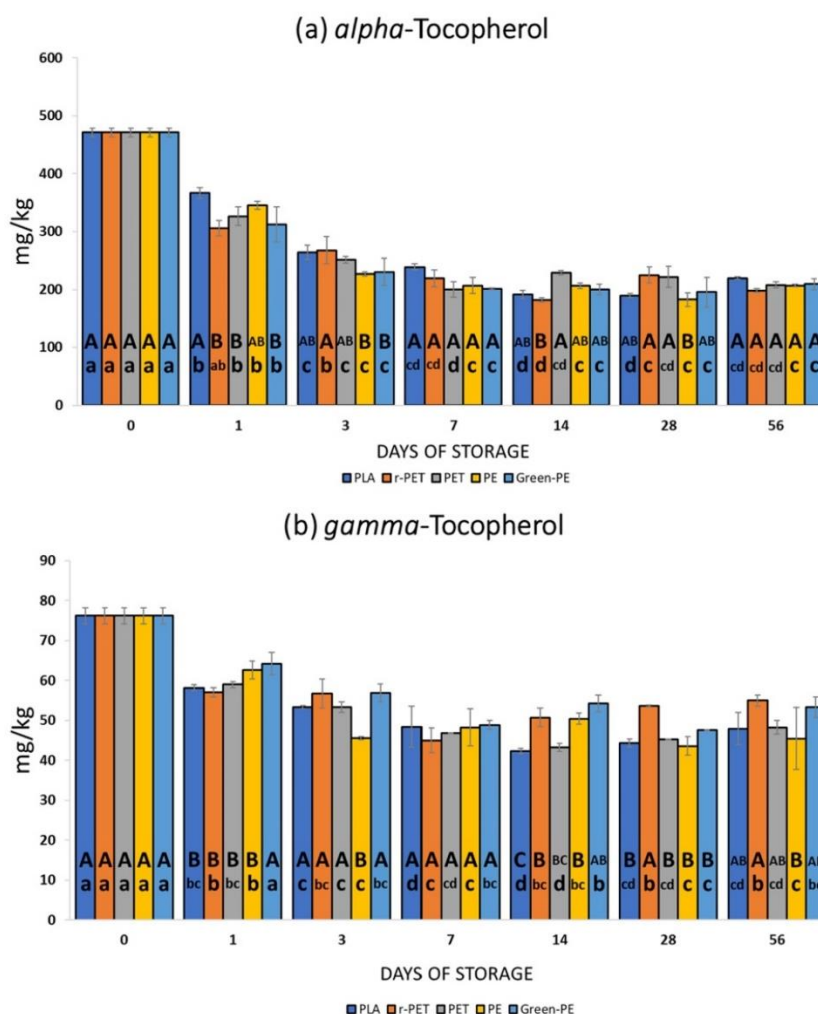


Fig. 4. Concentration (mg/kg) of (a)  $\alpha$ -tocopherol and (b)  $\gamma$ -tocopherol in sunflower oil during storage in different packaging materials. Different capital letters show significant differences between the plastic materials for each day. Different lowercase letters show significant differences for each material over the time of 56 days. Data are shown as mean  $\pm$  SD.

Table 1

Concentration of linoleic acyl groups, expressed as molar percentage, in sunflower oil during storage in different packaging materials. Different capital letters show significant differences between the plastic material for each day. Different lowercase letters show significant differences for each material over the time of 56 days. Data are shown as mean  $\pm$  SD.

| Samples  | Storage time (days)          |                              |                              |                              |                              |                              |                              |
|----------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|          | 0                            | 1                            | 3                            | 7                            | 14                           | 28                           | 56                           |
| PLA      | 55.5 $\pm$ 0.2 <sup>Aa</sup> | 55.6 $\pm$ 0.2 <sup>Aa</sup> | 55.5 $\pm$ 0.2 <sup>Aa</sup> | 55.5 $\pm$ 0.1 <sup>Aa</sup> | 55.4 $\pm$ 0.2 <sup>Aa</sup> | 55.1 $\pm$ 0.3 <sup>Aa</sup> | 55.5 $\pm$ 0.2 <sup>Aa</sup> |
| r-PET    | 55.5 $\pm$ 0.2 <sup>Aa</sup> | 55.5 $\pm$ 0.2 <sup>Aa</sup> | 55.4 $\pm$ 0.2 <sup>Aa</sup> | 55.5 $\pm$ 0.1 <sup>Aa</sup> | 55.6 $\pm$ 0.2 <sup>Aa</sup> | 55.2 $\pm$ 0.4 <sup>Aa</sup> | 55.3 $\pm$ 0.5 <sup>Aa</sup> |
| PET      | 55.5 $\pm$ 0.2 <sup>Aa</sup> | 55.5 $\pm$ 0.1 <sup>Aa</sup> | 55.4 $\pm$ 0.2 <sup>Aa</sup> | 55.5 $\pm$ 0.1 <sup>Aa</sup> | 55.6 $\pm$ 0.2 <sup>Aa</sup> | 55.6 $\pm$ 0.4 <sup>Aa</sup> | 55.3 $\pm$ 0.9 <sup>Aa</sup> |
| PE       | 55.5 $\pm$ 0.2 <sup>Aa</sup> | 55.2 $\pm$ 0.3 <sup>Aa</sup> | 55.4 $\pm$ 0.2 <sup>Aa</sup> | 55.4 $\pm$ 0.1 <sup>Aa</sup> | 55.3 $\pm$ 0.2 <sup>Aa</sup> | 55.4 $\pm$ 0.2 <sup>Aa</sup> | 55.4 $\pm$ 0.3 <sup>Aa</sup> |
| Green-PE | 55.5 $\pm$ 0.2 <sup>Aa</sup> | 55.5 $\pm$ 0.1 <sup>Aa</sup> | 55.6 $\pm$ 0.2 <sup>Aa</sup> | 55.7 $\pm$ 0.4 <sup>Aa</sup> | 55.5 $\pm$ 0.1 <sup>Aa</sup> | 55.6 $\pm$ 0.2 <sup>Aa</sup> | 55.4 $\pm$ 0.2 <sup>Aa</sup> |

the oxygen permeability in PLA is slightly higher than in PET, there were no significant differences between the oil samples packaged in PLA and PET and r-PET during the storage until day 28. However, sunflower oil in PE and Green-PE bottles showed much higher values of peroxides in comparison with the oil stored in other bottles. During the first 14 days an approximately two-fold higher peroxide value was obtained in oils

filled in PE and Green-PE bottles compared to the oil in the other bottles. This effect could be attributed to the higher oxygen permeability of PE and Green-PE compared to the other materials, offering a poor protection against lipid oxidation (Nisticò, 2020).

To confirm the results provided by the POV an  $^1\text{H}$  NMR study of the samples was performed. As expected, the  $^1\text{H}$  NMR results evidenced that



all samples contained hydroperoxides. In addition, in the spectra of all samples,  $^1\text{H}$  NMR signals appeared corresponding to hydroperoxy-*Z,E*-conjugated dienes. From these signals it was possible to determinate separately the total concentration of hydroperoxides and the concentration of hydroperoxy-*Z,E*-conjugated dienes, expressed as millimol per mol of triglyceride (mmol/mol TG).

Fig. 1b and c show the concentration of total hydroperoxides and hydroperoxy-*Z,E*-conjugated dienes in the differently packaged oil during storage for 56 days, respectively. It should be noted that the total hydroperoxide concentration (Fig. 1b) was twice as high as the concentration of hydroperoxy-*Z,E*-conjugated dienes (Fig. 1c). Therefore, it can be assumed that the oils contained other hydroperoxides different from the hydroperoxy-*Z,E*-conjugated dienes. In fact, Lee and Min (2010) showed that non-conjugated hydroperoxides formed during photosensitized oxidation of linoleic acid with  $^1\text{O}_2$ . In addition, by means of GC-MS, (*E*)-2-heptenal could be identified in all samples of the current study (section 3.3.2.). This aldehyde is a secondary oxidation product mainly derived from the cleavage of the C11-C12 bond of non-conjugated hydroperoxides formed by photosensitized oxidation of linoleic acyl groups. Therefore (*E*)-2-heptenal can be used as a marker of photosensitized oxidation and could also indicate the formation of non-conjugated hydroperoxides. Moreover, the same observation was found in the study of Min et al. (2003), as they detected (*E*)-2-heptenal only in the presence of a photosensitizer and light. With the detection of (*E*)-2-heptenal in the current study the missing hydroperoxides may be related to non-conjugated dienes.

The concentration of total hydroperoxides increased significantly over the period of 56 days in all bottles. The concentration of total hydroperoxides was two-fold and three-fold higher in PE and Green-PE samples ( $6.2 \pm 0.3$  and  $6.8 \pm 0.7$  mmol OOH/mol TG) than in PLA, r-PET, and PET ones ( $3.2 \pm 0.4$ ,  $1.9 \pm 0.2$  and  $1.8 \pm 0.1$  mmol OOH/mol TG respectively) after 56 days of storage (Fig. 1b). Regarding oil samples stored in PLA, similar concentrations of total hydroperoxides as for those stored in r-PET and PET bottles were observed until day 28 showing a significant increase of hydroperoxide concentration at the end of the storage. As expected, the concentration of hydroperoxy-*Z,E*-conjugated dienes showed a similar trend (see Fig. 1c).

Thus, according to these oxidation markers the oxidative stability of sunflower oil was higher when it was stored in PET and r-PET bottles, while Green-PE and PE showed the worst results. These results are in agreement with the POV. The variation of lipid oxidation between oils stored in r-PET, PET and PLA was low with a similar outcome after storage for 56 days at room temperature. Thus, the possibility for PLA to be used as an alternative packaging material for sunflower oil or other vegetable oils with similar composition should be considered.

### 3.3. Occurrence of secondary oxidation compounds

Further oxidative degradation of the oil causes the primary oxidation compounds to break into volatile and non-volatile compounds. These compounds are considered as secondary oxidation compounds and are the main reason for a change in aroma and off-flavor of the oil with advanced lipid oxidation (Grebenteuch et al., 2021). The secondary compounds comprise of aldehydes, ketones, alcohols, and acids and can be detected by methods such as the *p*-anisidine value or SPME-GC-MS (Frankel, 2005).

The *p*-anisidine value is a classic method to detect aldehydes in oxidized oils and is based on the reaction of their carbonyl groups with *p*-anisidine, to form a colored Schiff base which can be measured by UV spectroscopy (Skiera et al., 2012). It mainly reacts with 2-alkenals and 2, 4-alkadienals and is more sensitive to unsaturated aldehydes, which leads to a higher absorption in wavelength than that of saturated aldehydes (Yang & Boyle, 2016; Skiera et al., 2012). In the current study it is shown in Fig. S2 that the *p*-aV increased slightly, especially in PE and Green-PE after 56 days of storage; however, it remained stable with a value between 4 and 5. In a previous study from Guillén and Cabo

(2002) it was shown that under accelerated oxidation conditions ( $70^\circ\text{C}$ ), the *p*-aV in sunflower oil did not rise until a POV of almost 200 meq  $\text{O}_2/\text{kg}$  was reached. This could imply that the concentration of (*E*)-2-heptenal was not high enough in the samples to indicate a change in the *p*-aV over the storage time.

SPME-GC-MS provided information on the specific nature of volatile oxidation compounds generated as consequence of unsaturated acyl groups degradation during storage. Figs. 2 and 3 show the abundance of some of the volatile compounds detected in sunflower oil. Most volatile oxidation markers detected in this study were aldehydes, among which can be differentiated in saturated aldehydes such as hexanal, heptanal and nonanal and one unsaturated aldehyde, (*E*)-2-heptenal (Fig. 2). Likewise, an increase of some acids, such as pentanoic, octanoic and nonanoic acid was observed (Fig. 3) since they represent further decomposition products of hydroperoxides and can be considered as markers as well (Schaich, 2005).

Nonanal and heptanal derive from the breakdown of oleic acid, whereby hexanal and (*E*)-2-heptenal are generated by the oxidation from linoleic acid (Schaich, 2005; Grebenteuch et al., 2021). As above-commented, (*E*)-2-heptenal can be considered as a marker for photosensitized oxidation occurring in oils rich in linoleic acyl groups. Photosensitized oxidation may lead to the formation of non-conjugated hydroperoxides which may decompose to (*E*)-2-heptenal (Min et al., 2003; Lee & Min, 2010). With sunflower oil's main acyl groups being oleic and linoleic acyl groups, the observed aldehydes correspond to the findings in the literature. In the current study hexanal showed a three- to four-fold significant increase in the samples stored in PE and Green-PE after 56 days (Fig. 2a). In addition, (*E*)-2-heptenal showed a remarkable rise during the storage as well, while less pronounced increase was observed for heptanal and nonanal (Fig. 2b-d). On the other hand, PLA, r-PET and PET led to the lowest formation of hexanal, heptanal and (*E*)-2-heptenal in stored oil whereby the abundance of the latter was significantly less in PLA stored oil than in the oils stored in the other packaging materials after 28 and 56 days. The results provided by GC-MS showed that mostly saturated aldehydes were formed by the breakdown of hydroperoxides. Since *p*-aV is more sensitive to unsaturated aldehydes, this observation may explain the non-significant change of *p*-aV in the sunflower oil over the storage time.

Acids occur as secondary products in lipid oxidation from their respective aldehydes (Schaich, 2005). In the current study pentanoic acid, octanoic acid and nonanoic acid could be detected (Fig. 3). Although nonanoic acid can derive from both oleic acid and linoleic acid, octanoic acid is a marker for the breakdown of a hydroperoxide derived from oleic acid. Pentanoic acid is mainly formed from linoleic acid hydroperoxides (Schaich, 2005). In the current study, the overall abundance of acids observed in oil stored in PE and Green-PE was higher than in the oil packaged in the other plastic materials. While the results between PLA- r-PET and PET stored oil was similar, the abundance of pentanoic acid (Fig. 3a) in PLA packaged sunflower oil, however, was much lower than in the other bottles. Compared to r-PET, approximately 30% less pentanoic acid could be detected in sunflower oil stored after 56 days in PLA bottles, thereby corroborating the results for (*E*)-2-heptenal also showing the lowest abundance in oils stored in PLA bottles and indicating that PLA may be a good alternative as a packaging material for edible oils.

### 3.4. Stability of tocopherols in sunflower oil

Tocopherols naturally present in sunflower oil have antioxidative capability (Tena et al., 2019). The fresh sunflower oil had a concentration of  $471.4 \pm 7.5$  mg/kg  $\alpha$ -tocopherol and  $76.2 \pm 2.0$  mg/kg  $\gamma$ -tocopherol (Fig. 4), being these values aligned with those reported in the literature (Grilo et al., 2014). Over the time  $\alpha$ -tocopherol decreased by 53.4 – 57.9% in the current study, reading  $219.8 \pm 2.4$ ,  $198.2 \pm 3.2$ ,  $207.9 \pm 5.4$ ,  $206.8 \pm 2.2$  and  $209.3 \pm 9.3$  mg/kg ( $p < 0.0001$ ) for PLA, r-PET, PET, PE, and Green-PE respectively. Meanwhile,  $\gamma$ -tocopherol



decreased by 27.9–40.3% to a concentration of  $47.9 \pm 4.1$ ,  $54.9 \pm 1.4$ ,  $48.2 \pm 1.7$ ,  $45.6 \pm 7.7$  and  $53.3 \pm 2.6$  mg/kg for PLA, r-PET, PET, PE, and Green-PE, respectively. This could be due to that the tocopherols act as natural antioxidants inhibiting the progress of oxidative degradation. Like  $\alpha$ -tocopherol,  $\gamma$ -tocopherol stabilized after day 7 or in case of further degradation, this was not that high to be detected. Overall, tocopherols presented the same pattern of oxidation in all the samples. Therefore, it can be said that as no differences regarding the tocopherols content in sunflower oil could be detected between PLA, r-PET and PET bottles, PLA might be a renewable and non-fossil alternative to the conventional plastic bottles.

### 3.5. Migration of non-intentionally added substances from the bottle material

Packaging or food contact materials (FCMs) protect food from external contamination and preserve the nutritional value as well as the physical and sensory quality of food. According to Regulation (EC) No. 1935/2004 on materials and articles intended to come into contact with food, FCMs should neither release substances into foodstuffs in any amount that is suitable to induce a possible health risk, have an unreasonable change in their composition nor any alteration of their organoleptic properties. However, with the degradation of the material over time it is still possible for non-intentionally added substances (NIAS) to migrate from the packaging material into the oil. One of such NIAS is benzene. A maximum limit for benzene migrating into water intended for human consumption (tap water) was set to 1 µg/L (European Union, 2020), but to our knowledge no limit for vegetable oils has been established. The exact origin of benzene is still unclear; however, it is a product which derives from pyrolysis of PET resin, with higher amounts in r-PET due to contaminants and impurities forming during the recycling process (Thoden van Velzen et al., 2020). Giuffrida et al. (1999) investigated the occurrence of benzene in different vegetable oils packaged in aluminum boxes or PET boxes. Despite there being traces of benzene in a few samples stored in aluminum boxes, in all PET packaged oil samples the concentration of benzene was much higher. A study from Maalouly et al. (2013) was also able to detect benzene in sunflower oil samples stored in PET; however, under accelerated aging conditions, such as higher storage temperatures of 40 °C. In the current study, it was possible to detect and quantify benzene with SPME-GC/MS in sunflower oil stored in r-PET and PET after 28 and 56 days (Table 2). It should be noted that benzene concentration in oils stored in PLA, PE, or Green-PE was below the LOD. These results were expected since there has been only evidence of the release of benzene in PET materials so far, although this is the first time where this formation was investigated in PE, Green-PE and PLA to the best of our knowledge. After 56 days the concentration of benzene in oils packaged in PET and r-PET bottles was  $0.153 \pm 0.027$  µg/g and  $0.187 \pm 0.024$  µg/g, respectively. Even though the amount of oil consumed per day is much lower than the daily consumption of tap water and therefore a limit for benzene in sunflower oil cannot be claimed, consumers might prefer products free of NIAS. Thus, PLA may be an interesting option in this respect.

As commented above, PLA seems to be a good alternative to the conventional plastic bottles in order to store edible oils. Therefore, with the aim to investigate whether migration compounds from PLA could be detected in the oil, samples were analyzed by LC-MS in the current study. Several, migration tests with PLA were conducted in the previous years; however, most of them were investigated as plastic films and/or

under accelerated conditions of 60 °C with food simulants, such as ethanol as a fat simulant (Conn et al., 1995; Mutsuga et al., 2008; Ubéda et al., 2019; Aznar et al., 2019). PLA is a polymer consisting of cyclic and linear oligomers of lactic acid molecules. Most migration products from the PLA material were linear PLA oligomers which were already present in the material. It was concluded that cyclic oligomers did not migrate into the simulants because they reacted with the simulant and formed linear oligomers which then would migrate (Ubéda et al., 2019). The current study investigated whether these oligomers would migrate into the sunflower oil during storage. Thus, both PLA bottle material and oil samples were analyzed by LC-MS. The MS spectra were compared to those previously described in Ubéda et al. (2019). Two linear oligomers and four cyclic oligomers ranging from cyclic[LA]<sub>8</sub> to cyclic[LA]<sub>13</sub> were observed in the bottle material (Table S1). However, none of them or other oligomers were detected in the oil samples throughout the storage process, suggesting that the migration of these oligomers is inexistent or under the limit of detection. This provides, once again, evidence to consider PLA as a safe alternative sunflower oil packaging material under these specific storage conditions (short storage at room temperature), due to the absence of NIAS or any other unexpected FCM-derived substance along the 56 days of this study. Nonetheless, further longer storage studies should be performed to assess the safety of PLA for vegetable oils over time.

## 4. Conclusions

After storing the sunflower oil for up to 56 days at room temperature and under cold fluorescence light, it has been demonstrated that lipid oxidation was not prevented in the oils packaged in PE or Green-PE bottles to the same extent compared with the oils stored in PLA, PET and r-PET bottles. Likewise, sunflower oil packaged in PLA bottles showed similar results for primary oxidation compounds, such as peroxide value, total hydroperoxide and hydroperoxy Z,E-conjugated dienes as the oils stored in r-PET and PET bottles. Nevertheless, the formation of secondary lipid oxidation products, (E)-2-heptenal and pentanoic acid, was even shown to be less pronounced in sunflower oil stored in PLA than in r-PET or PET bottles. Therefore, PLA could be used as a renewable and non-fossil packaging material for edible oils, as it could prevent lipid oxidation of sunflower oil to a similar extent as r-PET and PET bottles. Moreover, benzene was not found in hazardous concentrations in any of the oil samples, being undetected for the ones stored in PLA bottles. All of this evidence suggests that using PLA bottles to store edible oils might be an alternative to r-PET or PET, providing oils free of NIAS which could have a better acceptance by the consumers. This study confirms the use of PLA as a plausible alternative to conventional plastic packaging such as PET for vegetables oils. Nevertheless, more research needs to be done on longer storage conditions, evaluating the light intensity and light transmission of the different packaging materials, their oxygen permeability as well as their color and thickness, to assess their impact on compound migration and oxidative stability.

### CRedit authorship contribution statement

**Martina Holler:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Jon Alberdi-Cedeño:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft & editing, Visualization. **Arturo Auñón-López:**

**Table 2**

Concentration of benzene in sunflower oil packaged in r-PET and PET after 28 and 56 days expressed as µg/g. Data are shown as mean ± SD.

|                 | PLA   |       | r-PET             |                   | PET               |                   | PE    |       | Green-PE |       |
|-----------------|-------|-------|-------------------|-------------------|-------------------|-------------------|-------|-------|----------|-------|
| Days of storage | 28    | 56    | 28                | 56                | 28                | 56                | 28    | 56    | 28       | 56    |
| Benzene (µg/g)  | < LOD | < LOD | $0.057 \pm 0.009$ | $0.187 \pm 0.024$ | $0.069 \pm 0.004$ | $0.153 \pm 0.027$ | < LOD | < LOD | < LOD    | < LOD |

LOD = Limit of detection, LOQ = Limit of quantification.



Methodology, Formal analysis, Data curation, Writing – review & editing. **Tobias Pointner**: Methodology, Formal analysis, Writing – review & editing. **Andrea Martínez-Yusta**: Writing – review & editing. **Jürgen König**: Writing – review & editing. **Marc Pignitter**: Conceptualization, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

#### Declaration of Competing Interest

The authors declare no conflicts of interest.

#### Data Availability

Data will be made available on request.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fpsl.2023.101051.

#### References

- Alberdi-Cedeño, J., Ibargoitia, M. L., Cristillo, G., Sopelana, P., & Guillén, M. D. (2017). A new methodology capable of characterizing most volatile and less volatile minor edible oils components in a single chromatographic run without solvents or reagents. Detection of new components. *Food Chemistry*, 221, 1135–1144. <https://doi.org/10.1016/j.foodchem.2016.11.046>
- Alberdi-Cedeño, J., Ibargoitia, M. L., & Guillén, M. D. (2019). Monitoring of minor compounds in corn oil oxidation by direct immersion solid phase microextraction-gas chromatography/mass spectrometry. New oil oxidation markers. *Food Chemistry*, 290, 286–294. <https://doi.org/10.1016/j.foodchem.2019.04.001>
- Aznar, M., Ubeda, S., Dreolin, N., & Nerin, C. (2019). Determination of non-volatile components of a biodegradable food packaging material based on polyester and polylactic acid (PLA) and its migration to food simulants. *Journal of Chromatography A*, 1583, 1–8. <https://doi.org/10.1016/j.chroma.2018.10.055>
- Bao, L., Dorgan, J. R., Knauss, D., Hait, S., Oliveira, N. S., & Maruccio, I. M. (2006). Gas permeation properties of poly (lactic acid) revisited. *Journal of Membrane Science*, 285(1–2), 166–172. <https://doi.org/10.1016/j.memsci.2006.08.021>
- Baumjohann, N., & Harns, D. (2015). Development of an LC-MS/MS method for studying migration characteristics of acetaldehyde in polyethylene terephthalate (PET)-packed mineral water. *Food Additives & Contaminants: Part A*, 32(9), 1552–1560. <https://doi.org/10.1080/19440049.2015.1075257>
- Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety*, 5(4), 169–186. <https://doi.org/10.1111/j.1541-4337.2006.00009.x>
- Codex Alimentarius Commission. (1999). Section 2. Codex standards for fats and oils from vegetable sources. <https://www.fao.org/3/y2774e/y2774e04.htm>
- Conn, R. E., Kolstad, J. J., Borzelleca, J. F., Dixler, D. S., Filer Jr, L. J., LaDu Jr, B. N., & Pariza, M. W. (1995). Safety assessment of polylactide (PLA) for use as a food-contact polymer. *Food and Chemical Toxicology*, 33(4), 273–283. [https://doi.org/10.1016/0278-6915\(94\)00145-E](https://doi.org/10.1016/0278-6915(94)00145-E)
- European Union. (2011). Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food Text with EEA relevance. Off. J. Eur. Union, 12, 1–89. <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32011R0010>
- European Union. (2020). Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the Quality of Water Intended for Human Consumption. Off. J. Eur. Union, 435, 1–62. <https://eur-lex.europa.eu/eli/dir/2020/2184/oj>
- European Parliament (2018). Plastic waste and recycling in the EU: facts and figures. [https://www.europarl.europa.eu/pdfs/news/expert/2018/12/story/20181212STO21610/20181212STO21610\\_en.pdf](https://www.europarl.europa.eu/pdfs/news/expert/2018/12/story/20181212STO21610/20181212STO21610_en.pdf)
- Frankel, E. N. (2005). *Lipid oxidation*. Elsevier. <https://www.sciencedirect.com/book/9780953194988/lipid-oxidation>
- Fruehwirth, S., Zehentner, S., Salimi, M., Steneder, S., Tiroch, J., Lieder, B., & Pignitter, M. (2020). In vitro digestion of grape seed oil inhibits phospholipid regulating effects of oxidized lipids. *Biomolecules*, 10(5), 708. <https://doi.org/10.3390/biom10050708>
- Giuffrida, R., Zerbo, A., & Clasadonte, M.T. (1999). Determination of benzene and toluene in vegetable oils by SPME/HPLC. Determination of Benzene and Toluene in Vegetable Oils by SPME/HPLC, 1000–1012. <https://digital.casalini.it/10.1400/55343>
- Grebenteuch, S., Kroh, L. W., Drusch, S., & Rohn, S. (2021). Formation of secondary and tertiary volatile compounds resulting from the lipid oxidation of rapeseed oil. *Foods*, 10(10), 2417. <https://doi.org/10.3390/foods10102417>
- Grilo, E. C., Costa, P. N., Gurgel, C. S. S., Beserra, A. F. D. L., Almeida, F. N. D. S., & Dimenstein, R. (2014). Alpha-tocopherol and gamma-tocopherol concentration in vegetable oils. *Food Science and Technology*, 34, 379–385. <https://doi.org/10.1590/S0101-20612014005000031>
- Grosshagauer, S., Steinschaden, R., & Pignitter, M. (2019). Strategies to increase the oxidative stability of cold pressed oils. *LWT*, 106, 72–77. <https://doi.org/10.1016/j.lwt.2019.02.046>
- 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(36), 10174–10184. <https://doi.org/10.1021/acs.jafc.9b03601>
- Guillén, M. D., & Cabo, N. (2002). Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chemistry*, 77(4), 503–510. [https://doi.org/10.1016/S0308-8146\(01\)00371-5](https://doi.org/10.1016/S0308-8146(01)00371-5)
- Guillén, M. D., Cabo, N., Ibargoitia, M. L., & Ruiz, A. (2005). Study of both sunflower oil and its headspace throughout the oxidation process. Occurrence in the headspace of toxic oxygenated aldehydes. *Journal of Agricultural and Food Chemistry*, 53(4), 1093–1101. <https://doi.org/10.1021/jf0489062>
- Guillén, M. D., & Ruiz, A. (2003). <sup>1</sup>H nuclear magnetic resonance as a fast tool for determining the composition of acyl chains in acylglycerol mixtures. *European Journal of Lipid Science and Technology*, 105(9), 502–507. <https://doi.org/10.1002/ejlt.200300799>
- Guillén, M. D., & Ruiz, A. (2005). Oxidation process of oils with high content of linoleic acyl groups and formation of toxic hydroperoxy and hydroxyalkenals. A study by <sup>1</sup>H nuclear magnetic resonance. *Journal of the Science of Food and Agriculture*, 85(14), 2413–2420. <https://doi.org/10.1002/jsfa.2273>
- Guillén, M. D., & Uriarte, P. S. (2012). Study by <sup>1</sup>H NMR spectroscopy of the evolution of extra virgin olive oil composition submitted to frying temperature in an industrial fryer for a prolonged period of time. *Food Chemistry*, 134(1), 162–172. <https://doi.org/10.1016/j.foodchem.2012.02.083>
- Guinault, A., Sollogoub, C., Domenek, S., Grandmontagne, A., & Ducruet, V. (2010). Influence of crystallinity on gas barrier and mechanical properties of PLA food packaging films. *International Journal of Material Forming*, 3(1), 603–606. <https://doi.org/10.1007/s12289-010-0842-9>
- Gunstone, F. (Ed.). (2011). Vegetable oils in food technology: composition, properties and uses. John Wiley & Sons. <https://doi.org/10.1002/9781144339925>
- Ha, J., Seo, D. W., Chen, X., Hwang, J. B., & Shim, Y. S. (2011). Determination of hexanal as an oxidative marker in vegetable oils using an automated dynamic headspace sampler coupled to a gas chromatograph/mass spectrometer. *Analytical Sciences*, 27(9). <https://doi.org/10.2116/analsci.27.873>
- Hu, K., Hyuan, Z., Ding, S., Dong, Y., & Yu, X. (2020). Investigation on food packaging polymers: Effects on vegetable oil oxidation. *Food Chemistry*, 315, Article 126299. <https://doi.org/10.1016/j.foodchem.2020.126299>
- Huyen, Z., Ding, S., Mao, X., Wu, C., & Yu, X. (2019). Effects of packaging materials on oxidative product formation in vegetable oils: Hydroperoxides and volatiles. *Food Packaging and Shelf Life*, 21, Article 100328. <https://doi.org/10.1016/j.fpsl.2019.100328>
- Katsara, K., Kenanakis, G., Viskadourakis, Z., & Papadakis, V. M. (2021). Polyethylene migration from food packaging on cheese detected by raman and infrared (ATR/FT-IR) spectroscopy. *Materials*, 14(14), 3872. <https://doi.org/10.3390/ma14143872>
- Lagaron, J. M., Cabedo, L., Cava, D., Feijoo, J. L., Gavara, R., & Gimenez, E. (2005). Improving packaged food quality and safety. Part 2: Nanocomposites. *Food Additives and Contaminants*, 22(10), 994–998. <https://doi.org/10.1080/02652030500239656>
- Laguette, M., Tenon, M., Bily, A., & Birtic, S. (2020). Toward a spatiotemporal model of oxidation in lipid dispersions: A hypothesis-driven review. *European Journal of Lipid Science and Technology*, 122(3), 1900209–1900218. <https://doi.org/10.1002/ejlt.201900209>
- Lee, J., & Min, D. B. (2010). Analysis of volatile compounds from chlorophyll photosensitized linoleic acid by headspace solid-phase microextraction (HS-SPME). *Food Science and Biotechnology*, 19(3), 611–616. <https://doi.org/10.1007/s10068-010-0086-y>
- Maalouty, J., Hayeck, N., Kassouf, A., Rutledge, D. N., & Ducruet, V. (2013). Chemometric tools to highlight possible migration of compounds from packaging to sunflower oils. *Journal of Agricultural and Food Chemistry*, 61(44), 10565–10573. <https://doi.org/10.1021/jf402888e>
- Martínez Yusta, A., Goicoechea, E., & Guillén, M. D. (2014). A review of thermo-oxidative degradation of food lipids studied by <sup>1</sup>H NMR spectroscopy: Influence of degradative conditions and food lipid nature. *Comprehensive Reviews in Food Science and Food Safety*, 13(5), 838–859. <https://doi.org/10.1111/1541-4337.12090>
- Martínez-Yusta, A., & Guillén, M. D. (2014). A study by <sup>1</sup>H 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. <https://doi.org/10.1016/j.foodres.2013.11.022>
- Min, D. B., Callison, A. L., & Lee, H. O. (2003). Singlet oxygen oxidation for 2-pentylfuran and 2-pentenylfuran formation in soybean oil. *Journal of Food Science*, 68(4), 1175–1178. <https://doi.org/10.1111/j.1365-2621.2003.tb09620.x>
- Mutsaers, M., Kawamura, Y., & Tanamoto, K. (2008). Migration of lactic acid, lactide and oligomers from polylactide food-contact materials. *Food Additives and Contaminants*, 25(10), 1283–1290. <https://doi.org/10.1080/02652030802017529>

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- Naser, A. Z., Deiab, I., & Darras, B. M. (2021). Poly (lactic acid) (PLA) and polyhydroxyalkanoates (PHAs), green alternatives to petroleum based plastics: A review. *RSC Advances*, 11(28), 17151–17196. <https://doi.org/10.1039/D1RA02390J>
- Nisticò, R. (2020). Polyethylene terephthalate (PET) in the packaging industry. *Polymer Testing*, 90, Article 106707. <https://doi.org/10.1016/j.polymertesting.2020.106707>
- 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(10), 2297–2305. <https://doi.org/10.1021/jf405736j>
- Rahman, M. H., & Bhoi, P. R. (2021). An overview of non-biodegradable bioplastics. *Journal of Cleaner Production*, 294, Article 126218. <https://doi.org/10.1016/j.jclepro.2021.126218>
- Ramezani, R. (2007). The effect of packaging materials and storage condition on the oxidative stability of refined sunflower oil. *Food Science and Technology Research*, 10(3), 350–354. <https://doi.org/10.3136/fstr.10.350>
- Schaich, K.M. (2005). Lipid oxidation: theoretical aspects. Bailey's industrial oil and fat products. <https://doi.org/10.1002/047167849X.bio067>
- Schaich, K. M. (2012). Thinking outside the classical chain reaction box of lipid oxidation. *Lipid Technology*, 24(3), 55–58. <https://doi.org/10.1002/lite.201200170>
- Shaikh, S., Yaqoob, M., & Aggarwal, P. (2021). An overview of biodegradable packaging in food industry. *Current Research in Food Science*, 4, 503–520. <https://doi.org/10.1016/j.crf.2021.07.005>
- Sharma, G. K., Madhura, C. V., & Arya, S. S. (1990). Interaction of plastic films with foods. II, Effect of polyethylene and polypropylene films on the stability of vegetable oils. *Journal of Food Science and Technology*, 27(6), 328–331.

### 4.2.2 Temperature-dependent antioxidative effects of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine fortification in polyunsaturated fatty acids-rich vegetable oils

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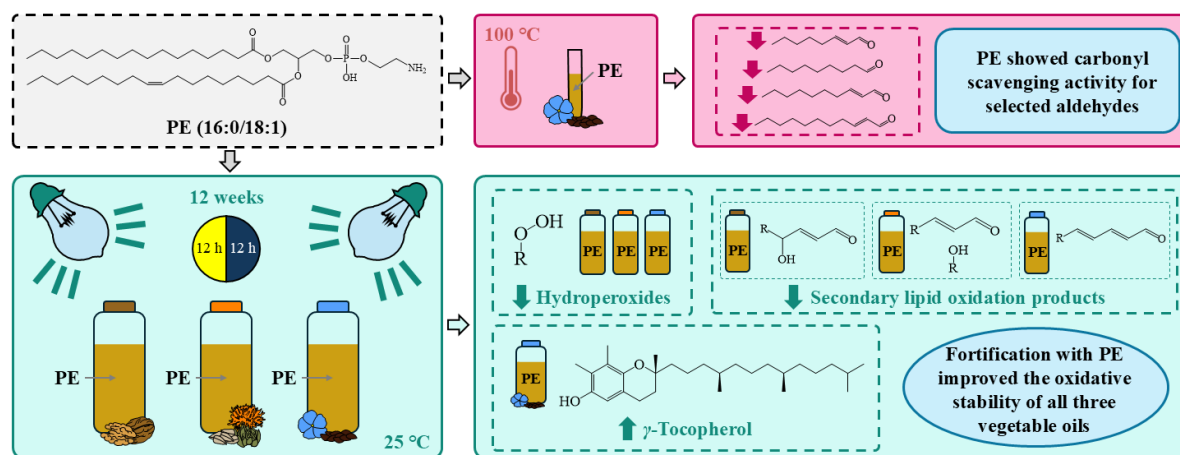
**Keywords:** Vegetable oils, Oxidative stability, Lipid carbonyls, Antioxidants, Aminophospholipids

**Status:** Submitted (to LWT)

**Contribution:** I helped through methodological support in oxidative stability testing, including Rancimat analysis and data evaluation. I participated in data interpretation, manuscript revision, reviewing and editing and participated in scientific discussions of the work.



## Graphical Abstract



## Highlights

- Phosphatidylethanolamine (PE) addition was evaluated as an antioxidant strategy
- PE fortification increased the oxidation induction time of three vegetable oils
- Fortified oils showed lower levels of oxidation during simulated household storage
- Fortification delayed  $\gamma$ -tocopherol consumption during storage of flaxseed oil
- PE may act as a carbonyl scavenger specifically during heating at 100 °C

## Abstract

Addition of aminophospholipids has previously shown antioxidative effects in edible oils, yet their impact on cold-pressed vegetable oils is still unexplored, despite their high susceptibility to oxidation. In this study, the potential of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (PE(16:0/18:1)) to act as an antioxidant and enhance the oxidative stability of three cold-pressed vegetable oils (walnut, safflower seed, and flaxseed) was evaluated at different temperatures. Fortification with 400 µg/g PE(16:0/18:1) resulted in an increased oxidation induction time under accelerated oxidizing conditions (100 °C) by means of 26.82%-50.12%, suggesting a longer shelf-life. Proton nuclear magnetic resonance (<sup>1</sup>H NMR) analysis of fortified oil samples stored up to 12 weeks in household-simulated conditions displayed reduced contents of lipid hydroperoxides as well as certain unsaturated aldehydes, evidencing an improved oxidative stability. Moreover, gas chromatography-mass spectrometry (GC-MS) analysis of highly thermally oxidized flaxseed oil (100 °C, 10 h) revealed a decreased content of specific lipid-derived carbonyls when PE(16:0/18:1) was added, suggesting its potential to act as carbonyl scavenger upon heating. Therefore, this study highlighted the potential of PE(16:0/18:1) as an antioxidant to increase the oxidative stability of vegetable oils in a heat-dependent manner, emphasizing the importance of considering different antioxidant activity mechanisms depending on the temperature conditions applied.

## 1. Introduction

Edible vegetable oils, specifically those rich in polyunsaturated fatty acids (PUFA), are highly valued for their nutritional benefits and health-promoting properties, including anti-inflammatory effects and protection against cardiovascular disease (Kapoor et al., 2021). However, their high PUFA content renders them prone to be easily affected by lipid oxidation during storage or thermal treatments, leading to the formation of off-flavors and toxic oxidation

compounds, and decreasing their nutritional value and quality (Vieira et al., 2017).

To overcome this challenge, the food industry has traditionally used synthetic antioxidants, which increase the oxidative stability of the oils and enhance their shelf-life (Liu et al., 2025). However, current safety concerns about their long-term consumption demand the use of natural solutions instead (Khezerlou et al., 2022). Hence, supplementation of oils with natural antioxidants, including vitamin E, flavonoids, and ascorbyl palmitate, is a common practice (Liu et al., 2025).

Lecithins, usually extracted from soybeans or egg yolk, are considered valuable natural products, with several applications in food due to their emulsifying and stabilizing properties (Zhao et al., 2022). Among them, aminophospholipids are particularly of interest, as they are known to exert antioxidant effects in lipid foods via different mechanisms, such as interaction with tocopherols or trapping of carbonyls, which are common secondary lipid oxidation products (Doert et al., 2017; Hidalgo & Zamora, 2017). For instance, Cui et al. (2015) described phosphatidylethanolamines to show antioxidative effects in bulk oils via reverse micelles formation, while Zhong et al. (2023) associated their addition with an improved oxidative stability in refined camellia seed oil.

Despite these findings, investigations on whether aminophospholipids fortification can be a promising strategy to improve the oxidative stability of cold-pressed, PUFA-rich vegetable oils, and whether these effects are temperature-dependent, are lacking. This understanding is essential to determine the proper application of this strategy. Therefore, this study investigated the impact of fortification with an aminophospholipid, specifically 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (PE(16:0/18:1)), on the oxidative stability of three cold-pressed vegetable oils, namely walnut (*Juglans regia* L.), safflower seed (*Carthamus tinctorius*

L.), and flaxseed (*Linum usitatissimum* L.) oils. These oils were selected due to their different PUFA composition (Al Surmi et al., 2015; Gutiérrez-Luna et al., 2022; Martínez & Maestri, 2008). Likewise, PE(16:0/18:1) was the aminophospholipid of choice, as one of the most abundant phospholipids in these oils (Burkhardt, 1970; Herchi et al., 2011; Zlatanov et al., 1999). We hypothesize that PE(16:0/18:1) fortification might improve the oxidative stability of these oils through distinct mechanisms depending on the temperature applied. Hence, experiments involving the storage of the oils under room temperature household conditions as well as accelerated oxidation tests were performed.

## **2. Materials and methods**

### *2.1. Materials and chemicals*

The three organic cold-pressed vegetable oils used in this study were purchased from Hagenthaler Ölmanufaktur (St. Andrä-Wördern, Austria), while the aminophospholipid PE(16:0/18:1), used for their fortification, was obtained from Sigma-Aldrich (Vienna, Austria). Details on the rest of chemicals used can be found in Method S1 of the Supplementary Information.

### *2.2. Study design*

Each vegetable oil was separated into two groups: a non-fortified control group and a fortified group in which 400 µg PE(16:0/18:1)/g oil was added. This concentration exceeds the amount of PE naturally present in the oils, while representing a common concentration for other natural antioxidants such as tocopherols (Savage et al., 1999). Freshly prepared samples were used to evaluate the influence of PE(16:0/18:1) addition on the stability of the oils under accelerated oxidizing conditions (section 2.3) as well as their different antioxidant capacities.



In addition, to evaluate whether PE(16:0/18:1) fortification has an impact on the oxidative stability of these oils under common household conditions, 80 g of each oil were stored in 250 mL clear recycled polyethylene terephthalate (rPET) bottles (Packari GmbH, Kirchbichl, Austria) and kept in a cabinet at room temperature ( $25 \pm 2$  °C), where they were exposed to cold fluorescent 12 h/12 h light/dark cycles for 12 weeks, as described by Pignitter et al. (2014). Experiments were done in quadruplicate, with four independent bottles per oil group. Aliquots of approximately 8 g were withdrawn on days 0, 1, 2, 3, 7, 14, 28, 56, 84 of storage, simulating consumer handling. These aliquots were then stored under argon atmosphere at -80 °C until further use.

### *2.3. Determination of oxidative stability at 100 °C by Rancimat analysis*

The stability of freshly prepared oil samples under accelerated oxidizing conditions was initially evaluated via Rancimat analysis, based on Aktar and Adal (2019) and AOCS (1995), allowing the determination of the oxidation induction time (OIT), a marker of oxidative stability and shelf-life. Hence, Rancimat vessels ( $24 \times 150$  mm) containing  $3.0 \pm 0.1$  g oil were connected to a Rancimat apparatus (Metrohm 892, Herisau, Switzerland), and subjected to an air rate of 20 L/h and a temperature of 100 °C until the OIT was detected.

### *2.4. Determination of peroxide value (POV) by International Dairy Federation (IDF) assay*

To evaluate the influence of PE(16:0/18:1) addition on the oils' oxidative stability during room temperature storage, hydroperoxides, as main primary lipid oxidation products, were determined in the oil samples from different storage days, using the IDF method (International Dairy Federation, 1991; Shantha & Decker, 1994), as described in Method S2.

### *2.5. Analysis of the progression of lipid oxidation by proton nuclear magnetic resonance ( $^1\text{H}$ NMR)*

The oil aliquots from the different storage days were analyzed with a Bruker Avance 400 spectrometer (Billerica, US) operating at 400 MHz. Briefly, 200  $\mu\text{L}$  sample were mixed with 400  $\mu\text{L}$  deuterated chloroform containing 0.03% tetramethylsilane as internal reference.  $^1\text{H}$  NMR spectra were acquired using the parameters described by Martínez-Yusta and Guillén (2019): spectral width 6410 Hz, pulse width  $90^\circ$ , acquisition time 4.8190 s, number of scans 64, and relaxation delay 3 s, with a total acquisition time of 8 min 38 s. The resulting spectra were plotted and analyzed in MestReNova 14.0 (Mestrelab Research, Santiago de Compostela, Spain), following Fourier transform, baseline correction and phase correction. Identification and quantification of the signals from different unsaturated acyl groups and various oxidation compounds were carried out according to previous studies (Alberdi-Cedeño et al., 2020; Martínez-Yusta et al., 2014), considering the proportional relation between the number of protons that generate the signals and their area.

### *2.6. Analysis of volatile oxidation compounds by solid phase microextraction followed by gas chromatography-mass spectrometry (SPME/GC-MS)*

Volatile compounds analysis was carried out in the safflower seed oil samples as performed by Alberdi-Cedeño et al. (2020), as described in Method S3. Results were compared between samples using a semi-quantitative approach, via area under the curve for one the main ion peaks of each compound (Table S2).

### *2.7. Determination of tocopherols by high performance liquid chromatography-diode array detection (HPLC-DAD)*

The content of vitamin E in the oil samples from selected storage days was analyzed as outlined by Pignitter et al. (2014), using  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol standard curves for quantification (Table S1), with *rac*-tocol as internal standard, as described in Method S4.

### *2.8. Analysis of free radical scavenging activity by diphenyl-1-picrylhydrazyl radical (DPPH) assay*

The radical scavenging activity of freshly prepared non-fortified and fortified oil samples was assessed as described previously by Goritschnig et al. (2020), both at room temperature and at 100 °C (see Method S5).

### *2.9. Analysis of ferric ion reducing power (FRAP)*

To test whether fortification with PE(16:0/18:1) may have an impact on the ferric reducing activity of the phenolic fraction of the oils, FRAP assay was carried out as in a previous study (Benzie & Strain, 1996). In short, phenolic extracts from fresh oil samples were obtained as described by Pointner et al. (2024), which were then processed according to Method S6.

### *2.10. Determination of the metal chelating potential*

The metal chelating properties of fresh non-fortified and fortified oil samples were evaluated according to Zhong et al. (2012), with modifications. Briefly, 50 mg oil were dissolved in 1 mL 2-propanol and 200  $\mu$ L resulting solution were mixed with 1740  $\mu$ L ethanol and 20  $\mu$ L 2 mmol/L iron(II) chloride solution. A total of 40  $\mu$ L 5 mmol/L ferrozine was then added. After thoroughly mixing, 200  $\mu$ L were transferred to a 96-well clear plate and left

standing for 10 min. The absorbance of the samples at 562 nm was subsequently measured and the chelation percentage was calculated as described in Eq. 1.

$$\text{Chelation (\%)} = 100 \cdot (1 - (\text{Abs}_{562\text{S}} - \text{Abs}_{562\text{B}})/\text{A}_{562\text{Ctrl}}) \quad (\text{Eq. 1})$$

where  $\text{A}_{562\text{S}}$ ,  $\text{A}_{562\text{B}}$ , and  $\text{A}_{562\text{Ctrl}}$  are the absorbances at 562 nm of a sample, a blank without ferrozine, and a control without sample, respectively.

### 2.11. Evaluation of carbonyl trapping capacity under heating conditions

To analyze the potential of PE(16:0/18:1) to trap lipid-derived carbonyls, three simple systems consisting of one aldehyde in combination with PE(16:0/18:1) were prepared, with deuterated chloroform as a matrix. Hence, stock solutions of 1.4 mmol/L PE(16:0/18:1), 6.4 mmol/L 4-hydroxy-(*E*)-2-nonenal, 5.6 mmol/L (*E,E*)-2,4-decadial, and 0.9 mmol/L acrolein were prepared, and PE(16:0/18:1) was mixed with each of the aldehydes in a PE:aldehyde molar ratio of 1:2, 1:5, and 1:5, for 4-hydroxy-(*E*)-2-nonenal, (*E,E*)-2,4-decadial, and acrolein, respectively. These ratios were based on the fact that one PE(16:0/18:1) molecule has been previously suggested to react with more than one carbonyl molecule (Goritschnig et al., 2020). The mixtures were diluted in deuterated chloroform until reaching 600  $\mu\text{L}$  and transferred to glass vials, where they were heated at 100  $^{\circ}\text{C}$  for 16 h. Afterwards, they were analyzed in a Bruker Avance 400 spectrometer as described in section 2.5, to determine their aldehyde content. In addition, control samples without PE were similarly processed and analyzed. Aldehyde signals were assigned according to Martínez-Yusta et al. (2014) as well as confirmed with standards. For quantification, standard curves for each aldehyde were used (Table S1).

Additionally, to evaluate whether PE(16:0/18:1) is able to trap carbonyls in oil matrices under heating conditions, freshly prepared non-fortified and fortified flaxseed oil samples were heated at 100  $^{\circ}\text{C}$  for 10 h, as the susceptibility of this oil to oxidation during



thermal treatments ensures its highly oxidized state (Sun et al., 2021). Afterwards, the heated oil samples were analyzed by SPME/GC-MS as described in section 2.6, to identify specific carbonyls that may be affected by PE fortification.

### *2.12. Statistical analysis*

All experiments were carried out at least in triplicate ( $n \geq 3$ ). GraphPad Prism 9.1.2 (Boston, MA, USA) was utilized to perform the statistical analyses corresponding to normality of the data, homoscedasticity, and statistical significance between sample groups ( $p < 0.05$ ), as described in Method S7.

## **3. Results and discussion**

### *3.1. Initial evaluation of the impact of PE(16:0/18:1) fortification on the oxidative stability of vegetable oils by Rancimat*

To evaluate whether PE(16:0/18:1) may represent a promising antioxidant to increase the oxidative stability of walnut, safflower seed, and flaxseed oils, the oxidation induction time (OIT) of their freshly prepared non-fortified and PE-fortified versions was determined at 100 °C using the Rancimat method. As shown in Fig. 1, the OIT values in the control oils were  $6.92 \pm 0.11$  h for walnut oil,  $6.79 \pm 0.88$  h for safflower seed oil, and  $4.24 \pm 0.05$  h for flaxseed oil. In the case of the latter two, the values were in line with previous research (Al Surmi et al., 2015; Varas Condori et al., 2020). Interestingly, non-fortified walnut oil showed higher OIT at 100 °C than many other conventional walnut oils analyzed prior to this study (Martínez & Maestri, 2008). Nevertheless, pre-existing evidence suggests a great variability in the oxidative stability of this oil depending on the walnut variety used for its extraction (Savage et al., 1999).

Regarding the fortified oils, the addition of PE(16:0/18:1) caused a significant increase ( $p < 0.01$ ) in the OIT of all three seed oils, describing mean increments of 36.22%, 26.82%, and 50.12% for walnut, safflower seed, and flaxseed oil, respectively. This finding strongly indicates that fortification with 400  $\mu\text{g/g}$  PE(16:0/18:1) improves the oxidative stability of these oils. A higher OIT represents a marker for an enhanced shelf-life, which entails several benefits in edible oils, such as better quality and reduced food waste, thereby being of interest for consumers and industries (Blasi & Cossignani, 2020; Varas Condori et al., 2020). Therefore, PE fortification may be a promising strategy to increase the shelf-life of these vegetable oils. In this sense, a previous study demonstrated the enhancing effect of a PE mix on the shelf-life of refined olive oil (Hidalgo et al., 2006). However, this is the first time, to our knowledge, that such effect is reported for cold-pressed, PUFA-rich vegetable oils, which are particularly prone to oxidation and might especially benefit from this strategy. Nevertheless, limitations on accelerated oxidation tests have been pointed out previously, as results under heating conditions cannot always be extrapolated to a room temperature setup (Kreps et al., 2021). Therefore, this study additionally evaluated the effects of PE(16:0/18:1) fortification during storage at room temperature, to determine whether the role of this phospholipid might be heat-dependent.

### *3.2. Oxidative stability of PE-fortified oils during 12 weeks of household storage conditions*

Since PE(16:0/18:1) fortification showed promising antioxidative effects on the oils under accelerated oxidative conditions, it was of interest to determine whether this effect translated to typical household storage. Hence, its impact during a 12-week storage period was investigated by assessing the content of lipid oxidation products and certain antioxidants in the oils.

### 3.2.1. Influence of PE fortification on primary lipid oxidation products

The determination of the POV has allowed the determination of hydroperoxides, main primary lipid oxidation compounds, for almost a century (Wheeler, 1932). Hence, the IDF assay, a spectrophotometric-based variant of this method, was used to analyze the POV in the oil samples (Fig. S1). A gradual increase throughout storage was observed for all oil groups, displaying total mean increments between the beginning and the end of the storage period ranging from 5.57 to 7.79 meq O<sub>2</sub>/kg ( $p < 0.0001$ ). Interestingly, the starting POV for walnut and flaxseed oils (Fig. S1A and C) was close to 0 meq O<sub>2</sub>/kg, while safflower seed oil exhibited a slightly oxidized state at the beginning of storage (Fig. S1B). Despite the gradual increase, all values reported were well below the established limit of 15 meq O<sub>2</sub>/kg for cold-pressed vegetable oils (Codex Alimentarius Commission, 1999). IDF assay did not reveal any significant changes ( $p > 0.05$ ) due to fortification with PE(16:0/18:1) in any of the three vegetable oils samples, regardless of the storage day in which they were taken, suggesting that this antioxidant does not have an impact on the content of hydroperoxides at room temperature, when supplemented in the selected concentration.

To confirm this, the degradation of polyunsaturated acyl groups and the formation of hydroperoxides in the oils were evaluated by <sup>1</sup>H NMR (Fig. 2). Linolenic acyl and linoleic acyl groups were detected in walnut and flaxseed oils, whereas only linoleic acyl groups could be observed in safflower seed oil. The content of these polyunsaturated fatty acyl groups was in agreement with previous fatty acid composition studies in these oils (Al Surmi et al., 2015; Gutiérrez-Luna et al., 2022; Martínez & Maestri, 2008). Non-fortified walnut oil did not undergo any significant change ( $p > 0.05$ ) on the molar percentage of linoleic and linolenic acyl groups during the whole storage period (Fig. 2A-B). Likewise, the overall effect of storage in PE-fortified walnut oil was negligible, as no significant difference ( $p > 0.05$ ) could be found

between the start and end points of storage. Furthermore, the presence of externally-added PE(16:0/18:1) did not significantly affect the concentration of polyunsaturated acyl groups of walnut oil in any of the different storage days ( $p > 0.05$ ). The absence of significant polyunsaturated acyl groups degradation for 12 weeks manifests a high oxidative stability for this oil, which can be further supported by the OIT value determined by Rancimat, which was, as explained in section 3.1, higher than the commonly reported values for this oil. Conversely, both non-fortified and PE-fortified safflower seed oils displayed a significant decrease ( $p < 0.01$ ) on their linoleic acyl groups after 4 weeks of storage, evidencing their oxidative degradation (Fig. 2C). As for flaxseed oil, both control and fortified versions showed a significantly decreased ( $p < 0.05$ ) linolenic acyl groups content by the end of storage in comparison to the fresh oil samples (Fig. 2D), while no overall significant effects ( $p > 0.05$ ) were observed on linoleic acyl groups (Fig. 2E). Indeed, linolenic acyl groups, the main acyl groups of flaxseed oil, have been reported to be especially prone to oxidation due to their higher number of double bonds (Brühl et al., 2022).

Fortification with PE(16:0/18:1) did not result in a significantly different ( $p > 0.05$ ) polyunsaturated acyl groups content for any of the three vegetable oils, in any of the storage days. Nevertheless, it should be pointed out that slight decreases on the content of these groups, even if not significant, may lead to an observable increase in hydroperoxides (Martínez-Yusta & Guillén, 2019). Therefore, the hydroperoxides content was additionally assessed by  $^1\text{H}$  NMR, as this technique allows the detection of signals specifically from hydroperoxide groups, with chemical shifts ranging from 8.3 to 8.9 ppm (Martínez-Yusta et al., 2014). This signal could be detected in walnut oil from storage day 2 onwards (Fig. 2F), while safflower seed oil samples already contained a detectable amount of hydroperoxides at the beginning of the storage period (Fig. 2G), which was in agreement with the POV determination. In contrast, the hydroperoxides signal could not be observed in flaxseed oil until day 14 (Fig. 2H). Once hydroperoxides were



detected, all three vegetable oils showed a gradual significant increase ( $p < 0.05$ ) on their content, regardless of their fortification state. However, on days 56 and 84, the PE-fortified oils displayed a significantly lower total hydroperoxides concentration ( $p < 0.01$ ) compared to the control oils. This apparent discrepancy with POV results may be explained by the high specificity of  $^1\text{H}$  NMR for particular nuclei (Barriuso et al., 2013), while the precision of iron-based spectrophotometric determinations might be partially hindered by a series of interferences, as explained by Bou et al. (2008). In addition, the detection of a higher number of significant differences on the content of oil hydroperoxides by  $^1\text{H}$  NMR in comparison to traditional POV determination has also been reported before (Holler et al., 2023). Nevertheless, in the present study, a positive significant correlation in hydroperoxide content between  $^1\text{H}$  NMR and the IDF assay was still observed ( $R = 0.7847$ ,  $p < 0.0001$ ).

Conjugated dienes represent another marker of the occurrence of primary lipid oxidation products, as hydroperoxide formation tends to involve the rearrangement of bis-allylic radicals forming conjugated double bond systems (Amft et al., 2023).  $^1\text{H}$  NMR allowed the detection of hydroperoxide-associated conjugated dienes (CD-OOH) in all oils, showing the formation of (*Z,E*)-CD-OOH and, to a lesser extent, (*E,E*)-CD-OOH, over time (Table 1). Similar to the total hydroperoxides determination, the contents of (*Z,E*)-CD-OOH in walnut and flaxseed oil as well as (*E,E*)-CD-OOH in flaxseed oil were significantly lower ( $p < 0.0001$ ) in the fortified oils by the end of storage, compared to their non-fortified counterparts. Alternatively, the lower total hydroperoxide amount in fortified safflower seed oil may be explained by the sum of (*Z,E*)-CD-OOH and (*E,E*)-CD-OOH rather than their individual values, showing a significantly lower amount ( $p < 0.01$ ) than the control oil samples.

The constant increase of hydroperoxides over time suggests the occurrence of the propagation phase of lipid oxidation, with the absence of termination reactions (Pignitter & Somoza, 2012). Hence, our findings evidence a limiting effect on the formation of primary lipid oxidation products by PE(16:0/18:1) fortification, albeit moderate enough not to significantly alter the POV obtained by the IDF assay.

### *3.2.2. Influence of PE fortification on secondary lipid oxidation products*

To further confirm the promising antioxidant role of PE(16:0/18:1) during household storage, the influence of PE fortification on the content of secondary lipid oxidation products was investigated. Different groups of carbonyl oxidation products were detected by means of  $^1\text{H}$  NMR (Table 2). Once again, walnut oil stood out for its high oxidative stability, as only the presence of a small amount of 4-hydroxy-(*E*)-2-alkenals ( $0.24 \pm 0.20$  mmol/mol triacylglycerols) could be observed on the last time point of the storage period. Alternatively, the presence of these aldehydes could not be demonstrated in the PE-fortified walnut oil, evidencing the oxidative stability enhancing properties of PE(16:0/18:1) addition. On the other hand, the spectra of the safflower seed oil samples reported the occurrence of n-alkanals after 4 weeks of storage, which became significantly more prominent ( $p < 0.05$ ) 8 weeks later. However, PE-fortified safflower seed oil did not show a significantly different ( $p > 0.05$ ) n-alkanals concentration in any storage day. As for flaxseed oil, the presence of four types of secondary oxidation products could be demonstrated: n-alkanals, which remained stable for most of the storage period; (*E*)-2-alkenals and (*E,E*)-2,4-alkadienals, detected from day 56 onwards; and 4-oxo-alkanals, only observable on day 84. Fortification of flaxseed oil with PE(16:0/18:1) did not have a significant impact ( $p > 0.05$ ) on the content of (*E*)-2-alkenals and 4-oxo-alkanals. However, it limited the content of (*E,E*)-2,4-alkadienals on day 56 and day 84 samples ( $p < 0.01$ ). Interestingly, fortification simultaneously increased ( $p < 0.05$ ) the content

of n-alkanals. Therefore, while the net effect of PE(16:0/18:1) on flaxseed oil was antioxidative due to (*E,E*)-2,4-alkadienals being the major secondary oxidation products detected, these results point towards the complexity of the role of this aminophospholipid during oil storage.

In order to determine whether the effects of PE(16:0/18:1) were specific on certain compounds, SPME/GC-MS analyses were performed. Safflower seed oil samples from early and late storage stages were chosen as the representative samples for this, aiming to observe possible significant effects not discernible by  $^1\text{H}$  NMR. Thus, several volatile oxidation products were identified in the safflower seed oil samples (Fig. 3A). A clear antioxidant effect due to fortification was observed, as the peak areas of all the compounds reported were significantly lower ( $p < 0.05$ ) in the fortified oil at some point of storage. Many volatile compounds evidenced this effect throughout the whole storage period, especially alcohols such as 3-methyl-1-butanol, 2-methyl-1-butanol, 1-pentanol, 2,3-butanediol, 1-hexanol or 2-octen-1-ol. Alternatively, other oxidation compounds, including pentanal, 2-heptanone, (*E*)-2-heptenal, octanal, 1-octen-3-ol,  $\gamma$ -caprolactone, and (*E*)-2-octenal, only showed significantly reduced ( $p < 0.05$ ) peak areas in comparison to non-fortified oils after at least 4 weeks. In agreement with the  $^1\text{H}$  NMR spectra, n-alkanals were the main group of secondary lipid oxidation products present in these oils, especially pentanal (Fig. 3B) and hexanal (Fig 3C). Both of them experienced a significant rise ( $p < 0.05$ ) in the non-fortified safflower seed oil on the last 4 weeks of storage, while remaining stable in the PE-fortified samples. The same was true for (*E*)-2-octenal (Fig. 3E), which, along with (*E*)-2-heptenal, conformed the major unsaturated secondary oxidation compounds detected. In contrast, the formation of (*E*)-2-heptenal was not completely avoided during the storage of fortified oil, although it managed to significantly delay it ( $p < 0.0001$ ) when comparing it to the control oil (Fig. 3D).

Overall, these results underline the positive effects of PE(16:0/18:1) fortification on the quality of the three vegetable oils studied during household storage, as it can contribute to delay the formation of off-flavors and the production of potentially toxic aldehydes such as (*E*)-2-alkenals or 4-hydroxy-(*E*)-2-alkenals (Vieira et al., 2017). Given the general antioxidative role of PE(16:0/18:1) on all volatile oxidation compounds detected in safflower oil, it can be likely seen as a consequence of the reduced hydroperoxides content (section 3.2.1) rather than a specific effect on individual oxidation compounds. Nevertheless, the occurrence of reactions between PE(16:0/18:1) and certain oxidation products, such as carbonyls, should not be completely ruled out, as the amino group from this phospholipid may be able to react with these compounds (Naudí et al., 2013).

### *3.2.3. Impact of PE addition on the content of tocopherols*

To evaluate whether fortification with PE(16:0/18:1) influences the concentration of other antioxidants, namely vitamin E, during the progression of lipid oxidation, the tocopherol content in the oil samples from selected storage days was analyzed (Fig. 5). Different isoforms were found depending on the oil ( $\gamma$ - and  $\delta$ - tocopherol in walnut oil,  $\alpha$ -tocopherol in safflower oil, and  $\gamma$ -tocopherol in flaxseed oil), in concentrations comparable to those reported in previous research (Aydeniz et al., 2014; Choo et al., 2007; Kafkas et al., 2017).  $^1\text{H}$  NMR allowed the determination of  $\gamma$ -tocopherol due to its characteristic singlet signal at 6.36 ppm, while  $\delta$ - and  $\alpha$ -tocopherol could only be determined by HPLC-DAD, since their signal in the NMR spectrum overlapped with other components in the oils. Fortification with PE(16:0/18:1) did not have a significant impact ( $p > 0.05$ ) on the  $\gamma$ -tocopherol content in walnut oil according to both techniques. In contrast,  $^1\text{H}$  NMR showed that fortification managed to significantly minimize ( $p < 0.05$ ) the consumption of  $\gamma$ -tocopherol in flaxseed oil samples from days 14, 28 and 56; nevertheless, the signal of this tocopherol could not be detected by the end of storage in both



non-fortified and fortified flaxseed oils (Fig. 5B). HPLC-DAD, conversely, allowed the quantification of a small amount of  $\gamma$ -tocopherol ( $27.7 \pm 1.0$  mg/kg) on day 84 PE-fortified flaxseed oil, evidencing that PE(16:0/18:1) partially preserved this form of tocopherol until the end of the storage period. Conversely, PE fortification did not cause a significant change on the contents of  $\delta$ -tocopherol in walnut oil and  $\alpha$ -tocopherol in safflower seed oil.

An extensive body of research suggests a synergism between the antioxidant activity of phosphatidylethanolamines and other primary antioxidants in vegetable oils, especially  $\alpha$ -tocopherol (Dziedzic & Hudson, 1984; Hidalgo et al., 2007; Takenaka et al., 2007). Some studies have even shown the regeneration of  $\alpha$ -tocopherol by phosphatidylethanolamine addition (Cui et al., 2015; Doert et al., 2017). Nevertheless, these studies observed this interaction effect specifically under heating conditions, which may explain the absence of a significant impact on  $\alpha$ -tocopherol content in safflower seed oil by PE(16:0/18:1) fortification. To our knowledge, there is no existing evidence that suggests the interaction between phosphatidylethanolamines and  $\gamma$ -tocopherol. Therefore, PE(16:0/18:1) might delay the consumption of  $\gamma$ -tocopherol in flaxseed oil by indirect mechanisms rather than their direct interaction (see section 3.3).

### *3.3. Antioxidant mechanisms of PE(16:0/18:1)*

Antioxidants in edible oils are known to act via different mechanisms, such as scavenging of free radicals (primary antioxidants), chelation of transition metal ions or reduction of ferric ions (secondary antioxidants), and trapping of carbonyls (tertiary antioxidants) (Hidalgo & Zamora, 2017; Liu et al., 2025). In the hope of elucidating the antioxidant mechanism by which PE(16:0/18:1) exerted its role during storage of the three vegetable oils, a series of antioxidant activity assays were carried out with fresh non-fortified

and fortified samples of each vegetable oil (Table 3). At 25 °C, although a different degree of DPPH scavenging activity was found among the different vegetable oils (e.g. higher scavenging activity in safflower seed oil compared to walnut and flaxseed oils), the presence of externally-added PE(16:0/18:1) did not significantly contribute ( $p > 0.05$ ) to this effect in any of them, thereby ruling out its role as primary antioxidant at the concentration used. Likewise, PE(16:0/18:1) did not enhance the ferric ion reducing and metal chelating properties of the oils, as no significant differences ( $p > 0.05$ ) between the PE-fortified and non-fortified oils were found by FRAP assay and metal chelating potential evaluation, respectively. As for tertiary antioxidant activity, the reduced content of several carbonyls in the fortified oil samples stored up to 12 weeks (Table 2 and Fig. 3) could indicate that PE(16:0/18:1) may exert its effect by trapping these carbonyls via carbonyl-amine reactions. However, as expressed in section 3.2.2, this is likely not the main mechanism taking place, as other volatile oxidation compounds, such as alcohols, as well as hydroperoxides, were equally affected. According to Saito and Ishihara (1997), phosphatidylethanolamines can act as hydroperoxide decomposers due to their ethanolamine group. Considering the results reported for the household storage samples, this is likely the main mechanism of the antioxidant effect observed, since the degradation of hydroperoxides by PE(16:0/18:1) would partially disrupt the formation of secondary oxidation products and hydroperoxide-derived free radicals, which would explain the preserving effect on  $\gamma$ -tocopherol in flaxseed oil.

It should be pointed out that, as expressed in section 3.2.3, PE(16:0/18:1) might interact with the oils' components differently depending on the temperature to which the samples were exposed. Therefore, it is reasonable to hypothesize that additional mechanisms might contribute to the OIT enhancing effect reported by Rancimat assay at 100 °C. In this sense, Goritschnig et al. (2020) showed that fortification with PE(16:0/18:1) increased the free radical scavenging activity of soybean oil specifically under heating conditions. Hence, DPPH

assay was carried out at 100 °C, to determine whether a similar situation took place in the oils of study. However, no such effect was observed in any of the oils (Table 3), likely due to the lower concentration used.

To evaluate additional carbonyl scavenging activity in heating conditions, PE:carbonyl reaction systems with PE(16:0/18:1) and different model aldehydes were first analyzed, after heating at 100 °C for 16 h (Fig. 5A-C). As shown, PE fortification significantly decreased ( $p < 0.05$ ) the concentration of 4-hydroxy-(*E*)-2-nonenal, to the point where it could not be detected, and acrolein, indicating that PE(16:0/18:1) reacts with them under these conditions. Conversely, the concentration of (*E,E*)-2,4-decadienal did not significantly change ( $p > 0.05$ ) with PE addition. Therefore, the capacity of PE(16:0/18:1) to scavenge selected carbonyls at 100 °C was demonstrated.

To test whether a similar effect could take place in oil, volatile oxidation compounds were analyzed in heated fortified and non-fortified samples at 100 °C. Flaxseed oil was selected for this evaluation, due to its high susceptibility to thermal oxidation (Sun et al., 2021). GC-MS analysis revealed, in the fortified flaxseed oil, a notable reduced content ( $p < 0.05$ ) of unsaturated aldehydes such as (*E*)-2-octenal, (*E*)-2-decenal, and (*E*)-2-undecenal, as well as nonanal (Fig. 5D-G), with the rest of the volatile compounds identified available in Table S3. This suggests that PE(16:0/18:1) acts as a tertiary antioxidant against these carbonyls during thermal treatments at 100 °C. In addition, the development of an orange color was observed specifically during heating of the PE-fortified flaxseed oil replicates (Fig. S2), which may be another marker of carbonyl-amine reactions taking place, since these have been linked to non-enzymatic browning in lipid-rich foods (Hidalgo & Zamora, 2000). Thus, future research should investigate specific potential structures derived from these reactions, to fully comprehend the antioxidative role of PE(16:0/18:1) and ensure its proper application.

#### 4. Conclusion

Fortification with PE(16:0/18:1) proved to be a promising strategy to enhance the oxidative stability, and thereby the shelf-life, of all three cold-pressed vegetable oils. While the effects on vegetable oils stored under household conditions were more likely due to PE(16:0/18:1) acting as a secondary antioxidant via hydroperoxides breakdown, heating of flaxseed oil at 100 °C revealed additional carbonyl scavenging activity in the PE-fortified samples, consequently resulting in the OIT increase determined by Rancimat analysis. This additional carbonyl trapping activity may be of special interest for frying oils, where toxic carbonyl compounds, such as (*E*)-2-alkenals, may form more readily during cooking. Therefore, this study evidenced, for the first time in cold-pressed vegetable oils, the temperature-dependent antioxidative role of PE(16:0/18:1). Thus, the importance of analyzing the effects of antioxidants in oils both under room temperature storage and accelerated oxidation conditions must be emphasized, as understanding their different mechanisms at play is crucial to ensure their correct application in the food industry. The impact of PE(16:0/18:1) fortification in frying oils should be studied by future research, to determine its reliability as a strategy to increase their oxidative stability. Lastly, upcoming studies should also investigate the formation of specific carbonyl-amine adducts derived from this antioxidative role and determine whether they are safe for consumption.

#### **CRedit authorship contribution statement**

**Arturo Auñon-Lopez:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review and editing. **Cristel Rial:** Formal analysis, Investigation, Visualization, Writing – review and editing. **Tobias Pointner:** Methodology, Writing – review and editing. **Marc Pignitter:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.



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## References

- Aktar, T., & Adal, E. (2019). Determining the Arrhenius kinetics of avocado oil: Oxidative stability under Rancimat test conditions. *Foods*, 8(7), Article 236. <https://doi.org/10.3390/foods8070236>.
- Al Surmi, N. Y., El Dengawi, R. A. H., Khalefa, A. H., & Yahia, N. (2015). Characteristics and oxidative stability of some safflower (*Carthamus tinctorius* L.). *Journal of Nutrition & Food Sciences*, 5(4), 1-6. <https://doi.org/10.4172/2155-9600.s14-001>.
- Alberdi-Cedeño, J., Ibarra, M. L., & Guillén, M. D. (2020). A global study by <sup>1</sup>H 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(4), Article 312. <https://doi.org/10.3390/antiox9040312>.
- Amft, J., Meissner, P. M., Steffen-Heins, A., Hasler, M., Stöckmann, H., Meynier, A., ... Schwarz, K. (2023). Interlaboratory study on lipid oxidation during accelerated storage trials with rapeseed and sunflower oil analyzed by conjugated dienes as primary oxidation products. *European Journal of Lipid Science and Technology*, 125(10), Article 2300067. <https://doi.org/10.1002/ejlt.202300067>.
- AOCS. (1995). American Oil Chemists' Society, Official Methods And Recommended Practices.
- Aydeniz, B., Güneşer, O., & Yılmaz, E. (2014). Physico-chemical, sensory and aromatic properties of cold press produced safflower oil. *Journal of the American Oil Chemists' Society*, 91(1), 99-110. <https://doi.org/10.1007/s11746-013-2355-4>.
- Barriuso, B., Astiasarán, I., & Ansorena, D. (2013). A review of analytical methods measuring lipid oxidation status in foods: a challenging task. *European Food Research and Technology*, 236(1), 1-15. <https://doi.org/10.1007/s00217-012-1866-9>.
- 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(1), 70-76. <https://doi.org/10.1006/abio.1996.0292>.
- Blasi, F., & Cossignani, L. (2020). An overview of natural extracts with antioxidant activity for the improvement of the oxidative stability and shelf life of edible oils. *Processes*, 8(8), Article 956. <https://doi.org/10.3390/pr8080956>.
- Bou, R., Codony, R., Tres, A., Decker, E. A., & Guardiola, F. (2008). Determination of hydroperoxides in foods and biological samples by the ferrous oxidation-xylenol orange method: a review of the factors that influence the method's performance. *Analytical Biochemistry*, 377(1), 1-15. <https://doi.org/10.1016/j.ab.2008.02.029>.
- Brühl, L., Bonte, A., N'Diaye, K., & Matthäus, B. (2022). Oxidation of cyclo-lipo peptides in linseed oils during storage. *European Journal of Lipid Science and Technology*, 124(12), Article 2200137. <https://doi.org/10.1002/ejlt.202200137>.
- Burkhardt, H. J. (1970). The phosphatides of safflower seeds involved in color formation occurring in extracted and heated crude oils. *Journal of the American Oil Chemists' Society*, 47(2), 69-72. <https://doi.org/10.1007/bf02541461>.
- Choo, W.-S., Birch, J., & Dufour, J.-P. (2007). Physicochemical and quality characteristics of cold-pressed flaxseed oils. *Journal of Food Composition and Analysis*, 20(3-4), 202-211. <https://doi.org/10.1016/j.jfca.2006.12.002>.
- Codex Alimentarius Commission. (1999). Section 2. Codex standards for fats and oils from vegetable sources.
- \*Cui, L., McClements, D. J., & Decker, E. A. (2015). Impact of phosphatidylethanolamine on the antioxidant activity of  $\alpha$ -tocopherol and trolox in bulk oil. *Journal of Agricultural and Food Chemistry*, 63(12), 3288-3294. <https://doi.org/10.1021/acs.jafc.5b00243>.
- \*Doert, M., Krüger, S., Morlock, G. E., & Kroh, L. W. (2017). Synergistic effect of lecithins for tocopherols: formation and antioxidant effect of the phosphatidylethanolamine-L-ascorbic acid condensate. *European Food Research and Technology*, 243(4), 583-596. <https://doi.org/10.1007/s00217-016-2768-z>.
- Dziedzic, S. Z., & Hudson, B. J. F. (1984). Phosphatidyl ethanolamine as a synergist for primary antioxidants in edible oils. *Journal of the American Oil Chemists' Society*, 61(6), 1042-1045. <https://doi.org/10.1007/bf02636214>.
- \*Goritschnig, J., Tadas, K., König, J., & Pignitter, M. (2020). Free radical scavenging activity of carbonyl-amine adducts formed in soybean oil fortified with phosphatidylethanolamine. *Molecules*, 25(2), Article 373. <https://doi.org/10.3390/molecules25020373>.
- Gutiérrez-Luna, K., Ansorena, D., & Astiasarán, I. (2022). Fatty acid profile, sterols, and squalene content comparison between two conventional (olive oil and linseed oil) and three non-conventional vegetable oils (echium oil, hempseed oil, and moringa oil). *Journal of Food Science*, 87(4), 1489-1499. <https://doi.org/10.1111/1750-3841.16111>.
- Herchi, W., Sakouhi, F., Khaled, S., Xiong, Y., Boukhchina, S., Kallel, H., & Curtis, J. M. (2011). Characterisation of the glycerophospholipid fraction in flaxseed oil using liquid chromatography-mass spectrometry. *Food Chemistry*, 129(2), 437-442. <https://doi.org/10.1016/j.foodchem.2011.04.096>.
- Hidalgo, F. J., León, M. M., & Zamora, R. (2006). Antioxidative activity of amino phospholipids and phospholipid/amino acid mixtures in edible oils as determined by the Rancimat method. *Journal of Agricultural and Food Chemistry*, 54(15), 5461-5467. <https://doi.org/10.1021/jf060848s>.
- Hidalgo, F. J., León, M. M., & Zamora, R. (2007). Effect of tocopherols in the antioxidative activity of oxidized lipid-amine reaction products. *Journal of Agricultural and Food Chemistry*, 55(11), 4436-4442. <https://doi.org/10.1021/jf070119s>.
- Hidalgo, F. J., & Zamora, R. (2000). The role of lipids in nonenzymatic browning. *Grasas Aceites*, 51(1-2). <https://doi.org/10.3989/gya.2000.v51.i1-2.405>.
- \*Hidalgo, F. J., & Zamora, R. (2017). Food processing antioxidants. In *Advances in Food and Nutrition Research* (pp. 31-64): Elsevier.
- Holler, M., Alberdi-Cedeño, J., Auñón-López, 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. <https://doi.org/10.1016/j.fpsl.2023.101051>.
- International Dairy Federation. (1991). Standard 74A.1991. Anhydrous milkfat: determination of peroxide value. Square Vergote 41, Brussels, Belgium.
- Kafkas, E., Burgut, A., Özcan, H., Özcan, A., Sutyemez, M., Kafkas, S., & Türems, N. (2017). Fatty acid, total phenol and tocopherol profiles of some walnut cultivars: A comparative study. *Food Science & Nutrition*, 08(12), 1074-1084. <https://doi.org/10.4236/fns.2017.812079>.
- Kapoor, B., Kapoor, D., Gautam, S., Singh, R., & Bhardwaj, S. (2021). Dietary polyunsaturated fatty acids (PUFAs): Uses and potential health benefits. *Current Nutrition Research*, 10(3), 232-242. <https://doi.org/10.1007/s13668-021-00363-3>.
- Khezerlou, A., Akhlaghi, A. P., Alizadeh, A. M., Dehghan, P., & Maleki, P. (2022). Alarming impact of the excessive use of tert-butylhydroquinone in food products: A narrative review. *Toxicology Reports*, 9, 1066-1075. <https://doi.org/10.1016/j.toxrep.2022.04.027>.
- Kreps, F., Dubaj, T., & Krepsová, Z. (2021). Accelerated oxidation method and simple kinetic model for predicting thermooxidative stability of edible oils under storage conditions. *Food Packaging and Shelf Life*, 29, Article 100739. <https://doi.org/10.1016/j.fpsl.2021.100739>.
- Liu, X., Zheng, Z., & Liu, Y. (2025). Lipophilic antioxidants in edible oils: Mechanisms, applications and interactions. *Food Research International*, 200, Article 115423. <https://doi.org/10.1016/j.foodres.2024.115423>.
- \*Martínez-Yusta, A., Goicoechea, E., & Guillén, M. D. (2014). A review of thermo-oxidative degradation of food lipids studied by <sup>1</sup>H NMR spectroscopy: Influence of degradative conditions and food lipid nature. *Comprehensive Reviews in Food Science and Food Safety*, 13(5), 838-859. <https://doi.org/10.1111/1541-4337.12090>.
- Martínez-Yusta, A., & Guillén, M. D. (2019). Enrichment of sunflower oil with  $\gamma$ -tocopherol. Study by <sup>1</sup>H NMR of its effect under accelerated storage conditions. *European Journal of Lipid Science and Technology*, 121(6), 1800457. <https://doi.org/10.1002/ejlt.201800457>.

- Martínez, M. L., & Maestri, D. M. (2008). Oil chemical variation in walnut (*Juglans regia* L.) genotypes grown in Argentina. *European Journal of Lipid Science and Technology*, 110(12), 1183-1189. <https://doi.org/10.1002/ejlt.200800121>.
- Naudí, A., Jové, M., Ayala, V., Cabré, R., Portero-Otín, M., & Pamplona, R. (2013). Non-enzymatic modification of aminophospholipids by carbonyl-amine reactions. *International Journal of Molecular Sciences*, 14(2), 3285-3313. <https://doi.org/10.3390/ijms14023285>.
- Pignitter, M., & Somoza, V. (2012). Critical evaluation of methods for the measurement of oxidative rancidity in vegetable oils. *Journal of Food and Drug Analysis*, 20(4), Article 6. <https://doi.org/10.38212/2224-6614.2024>.
- 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(10), 2297-2305. <https://doi.org/10.1021/jf405736j>.
- Pointner, T., Rauh, K., Auñón-Lopez, A., Kostadinović Veličkowska, S., Mitrev, S., Arsov, E., & Pignitter, M. (2024). Comprehensive analysis of oxidative stability and nutritional values of germinated linseed and sunflower seed oil. *Food Chemistry*, 454, Article 139790. <https://doi.org/10.1016/j.foodchem.2024.139790>.
- Saito, H., & Ishihara, K. (1997). Antioxidant activity and active sites of phospholipids as antioxidants. *Journal of the American Oil Chemists' Society*, 74(12), 1531-1536. <https://doi.org/10.1007/s11746-997-0072-6>.
- Savage, G. P., Dutta, P. C., & McNeil, D. L. (1999). Fatty acid and tocopherol contents and oxidative stability of walnut oils. *Journal of the American Oil Chemists' Society*, 76(9), 1059-1063. <https://doi.org/10.1007/s11746-999-0204-2>.
- Shantha, N. C., & Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC INTERNATIONAL*, 77(2), 421-424. <https://doi.org/10.1093/jaoac/77.2.421>.
- Sun, X., Wang, Y., Li, H., Zhou, J., Han, J., & Wei, C. (2021). Changes in the volatile profile, fatty acid composition and oxidative stability of flaxseed oil during heating at different temperatures. *LWT - Food Science and Technology*, 151, Article 112137. <https://doi.org/10.1016/j.lwt.2021.112137>.
- Takenaka, A., Hosokawa, M., & Miyashita, K. (2007). Unsaturated phosphatidylethanolamine as effective synergist in combination with  $\alpha$ -tocopherol. *Journal of Oleo Science*, 56(10), 511-516. <https://doi.org/10.5650/jos.56.511>.
- 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. <https://doi.org/10.1016/j.foodchem.2019.125327>.
- Vieira, S. A., Zhang, G., & Decker, E. A. (2017). Biological implications of lipid oxidation products. *Journal of the American Oil Chemists' Society*, 94(3), 339-351. <https://doi.org/10.1007/s11746-017-2958-2>.
- Wheeler, D. H. (1932). Peroxide formation as a measure of autoxidative deterioration. *Journal of the American Oil Chemists' Society*, 9(4), 89-97. <https://doi.org/10.1007/bf02553782>.
- Zhao, F., Li, R., Liu, Y., & Chen, H. (2022). Perspectives on lecithin from egg yolk: Extraction, physicochemical properties, modification, and applications. *Frontiers in Nutrition*, 9, Article 1082671. <https://doi.org/10.3389/fnut.2022.1082671>.
- Zhong, J. J., Ma, L., Sun, Y. L., Zhong, H. Y., & Zhou, B. (2023). Effect of phosphatidylethanolamine and phosphatidylserine on antioxidant capacity, oxidative stability and color reversion of camellia seed oil. *Grasas Aceites*, 74(2), Article e500. <https://doi.org/10.3989/gya.0334221>.
- Zhong, Y., Ma, C.-M., & Shahidi, F. (2012). Antioxidant and antiviral activities of lipophilic epigallocatechin gallate (EGCG) derivatives. *Journal of Functional Foods*, 4(1), 87-93. <https://doi.org/10.1016/j.jff.2011.08.003>.
- Zlatanov, M., Ivanov, S., & Aitzetmüller, K. (1999). Phospholipid and fatty acid composition of Bulgarian nut oils. *Fett/Lipid*, 101(11), 437-439. [https://doi.org/10.1002/\(SICI\)1521-4133\(199911\)101:11<437::AID-LIPI437>3.0.CO;2-T](https://doi.org/10.1002/(SICI)1521-4133(199911)101:11<437::AID-LIPI437>3.0.CO;2-T).

**Table 1.** Concentration of hydroperoxy-conjugated dienes (CD-OOH) in non-fortified (control) and PE-fortified oils stored under household conditions.

| Storage day | Fortification state | Walnut oil                 |                            | Safflower seed oil               |                            | Flaxseed oil               |                            |
|-------------|---------------------|----------------------------|----------------------------|----------------------------------|----------------------------|----------------------------|----------------------------|
|             |                     | (Z,E)-CD-OOH (mmol/mol TG) | (E,E)-CD-OOH (mmol/mol TG) | (Z,E)-CD-OOH (mmol/mol TG)       | (E,E)-CD-OOH (mmol/mol TG) | (Z,E)-CD-OOH (mmol/mol TG) | (E,E)-CD-OOH (mmol/mol TG) |
| Day 0       | Control             | ND                         | ND                         | 2.30 ± 0.25 <sup>a</sup>         | ND                         | 1.23 ± 0.25 <sup>a</sup>   | ND                         |
|             | PE-fortified        | ND                         | ND                         | 2.55 ± 0.20 <sup>A</sup>         | ND                         | 1.65 ± 0.13 <sup>A</sup>   | ND                         |
| Day 1       | Control             | ND                         | ND                         | 2.97 ± 0.17 <sup>a</sup>         | ND                         | 1.52 ± 0.70 <sup>a</sup>   | ND                         |
|             | PE-fortified        | ND                         | ND                         | 3.38 ± 0.33 <sup>AB</sup>        | ND                         | 1.91 ± 0.56 <sup>A</sup>   | ND                         |
| Day 2       | Control             | 0.48 ± 0.01 <sup>a</sup>   | ND                         | 3.82 ± 0.18 <sup>b</sup>         | ND                         | 1.66 ± 0.45 <sup>a</sup>   | ND                         |
|             | PE-fortified        | 0.53 ± 0.22 <sup>A</sup>   | ND                         | 4.48 ± 0.46 <sup>BC</sup>        | ND                         | 1.86 ± 0.40 <sup>A</sup>   | ND                         |
| Day 3       | Control             | 0.77 ± 0.26 <sup>a</sup>   | ND                         | 4.78 ± 0.31 <sup>b</sup>         | ND                         | 1.68 ± 0.30 <sup>a</sup>   | ND                         |
|             | PE-fortified        | 1.04 ± 0.24 <sup>A</sup>   | ND                         | 5.36 ± 0.61 <sup>C</sup>         | ND                         | 2.29 ± 0.11 <sup>A</sup>   | ND                         |
| Day 7       | Control             | 1.45 ± 0.20 <sup>ab</sup>  | ND                         | 8.39 ± 0.26 <sup>c</sup>         | 0.25 ± 0.15 <sup>a</sup>   | 2.61 ± 0.22 <sup>a</sup>   | ND                         |
|             | PE-fortified        | 1.70 ± 0.02 <sup>AB</sup>  | ND                         | 9.45 ± 0.09 <sup>D*</sup>        | 0.40 ± 0.22 <sup>A</sup>   | 2.86 ± 0.38 <sup>AB</sup>  | ND                         |
| Day 14      | Control             | 2.73 ± 0.21 <sup>b</sup>   | ND                         | 15.80 ± 0.03 <sup>d</sup>        | 0.43 ± 0.27 <sup>a</sup>   | 4.51 ± 0.73 <sup>b</sup>   | ND                         |
|             | PE-fortified        | 2.84 ± 0.23 <sup>B</sup>   | ND                         | 16.42 ± 0.21 <sup>E</sup>        | 0.46 ± 0.23 <sup>A</sup>   | 4.28 ± 0.31 <sup>B</sup>   | ND                         |
| Day 28      | Control             | 5.64 ± 0.27 <sup>c</sup>   | 0.31 ± 0.35 <sup>a</sup>   | 30.57 ± 0.27 <sup>e</sup>        | 1.51 ± 0.20 <sup>b</sup>   | 9.65 ± 0.61 <sup>c</sup>   | ND                         |
|             | PE-fortified        | 5.77 ± 0.22 <sup>C</sup>   | 0.58 ± 0.25 <sup>A</sup>   | 29.31 ± 2.40 <sup>E</sup>        | 1.57 ± 0.32 <sup>B</sup>   | 8.39 ± 0.64 <sup>C</sup>   | ND                         |
| Day 56      | Control             | 13.43 ± 1.23 <sup>d</sup>  | 1.13 ± 0.53 <sup>a</sup>   | 62.32 ± 0.49 <sup>f</sup>        | 3.56 ± 0.27 <sup>c</sup>   | 24.87 ± 1.01 <sup>d</sup>  | 1.21 ± 0.01 <sup>a</sup>   |
|             | PE-fortified        | 12.26 ± 0.62 <sup>D</sup>  | 1.28 ± 0.11 <sup>A</sup>   | 49.70 ± 13.45 <sup>ABCEDEF</sup> | 2.84 ± 1.13 <sup>C</sup>   | 20.70 ± 1.31 <sup>D*</sup> | 0.76 ± 0.04 <sup>A*</sup>  |
| Day 84      | Control             | 24.17 ± 2.22 <sup>e</sup>  | 3.88 ± 0.93 <sup>b</sup>   | 94.67 ± 1.00 <sup>g</sup>        | 7.06 ± 0.26 <sup>d</sup>   | 51.00 ± 1.11 <sup>e</sup>  | 6.11 ± 0.17 <sup>b</sup>   |
|             | PE-fortified        | 20.77 ± 0.64 <sup>E*</sup> | 3.20 ± 0.60 <sup>B</sup>   | 84.81 ± 3.10 <sup>F</sup>        | 6.53 ± 0.58 <sup>D</sup>   | 41.03 ± 0.31 <sup>E*</sup> | 3.46 ± 0.17 <sup>B*</sup>  |

Data is presented as mean ± SD (n ≥ 3). Different superscript lower-case letters (a, b, c, d, e, f, g) within each column indicate statistically significant difference (p < 0.05) between storage days for the control oils, while different upper-case letters (A, B, C, D, E, F) show statistically significant difference (p < 0.05) between storage days for the fortified oils. Asterisks (\*) on the values of the fortified oils express a statistically significant difference (p < 0.05) compared to their control counterpart on the same storage day. Significant differences were determined by two-way analysis of variance (ANOVA) or Welch's ANOVA, followed by Tukey's or Šidák's post hoc test. TG: triacylglycerol.

**Table 2.** Concentration of secondary lipid oxidation products in non-fortified (control) and PE-fortified oils stored under household conditions.

| Storage day | Fortification state | Walnut oil                             | Safflower seed oil        |                              | Flaxseed oil                        |                            |                              |
|-------------|---------------------|----------------------------------------|---------------------------|------------------------------|-------------------------------------|----------------------------|------------------------------|
|             |                     | 4-hydroxy-(E)-2-alkenals (mmol/mol TG) | n-alkanals (mmol/mol TG)  | (E)-2-alkenals (mmol/mol TG) | (E,E)-2,4-alkadienals (mmol/mol TG) | n-alkanals (mmol/mol TG)   | 4-oxo-alkanals (mmol/mol TG) |
| Day 0       | Control             | ND                                     | ND                        | ND                           | ND                                  | 0.23 ± 0.02 <sup>a</sup>   | ND                           |
|             | PE-fortified        | ND                                     | ND                        | ND                           | ND                                  | 0.24 ± 0.03 <sup>A</sup>   | ND                           |
| Day 1       | Control             | ND                                     | ND                        | ND                           | ND                                  | 0.16 ± 0.01 <sup>a</sup>   | ND                           |
|             | PE-fortified        | ND                                     | ND                        | ND                           | ND                                  | 0.25 ± 0.04 <sup>A</sup>   | ND                           |
| Day 2       | Control             | ND                                     | ND                        | ND                           | ND                                  | 0.20 ± 0.09 <sup>a</sup>   | ND                           |
|             | PE-fortified        | ND                                     | ND                        | ND                           | ND                                  | 0.22 ± 0.03 <sup>A</sup>   | ND                           |
| Day 3       | Control             | ND                                     | ND                        | ND                           | ND                                  | 0.22 ± 0.07 <sup>a</sup>   | ND                           |
|             | PE-fortified        | ND                                     | ND                        | ND                           | ND                                  | 0.27 ± 0.03 <sup>A</sup>   | ND                           |
| Day 7       | Control             | ND                                     | ND                        | ND                           | ND                                  | 0.24 ± 0.03 <sup>a</sup>   | ND                           |
|             | PE-fortified        | ND                                     | ND                        | ND                           | ND                                  | 0.22 ± 0.09 <sup>A</sup>   | ND                           |
| Day 14      | Control             | ND                                     | ND                        | ND                           | ND                                  | 0.17 ± 0.04 <sup>a</sup>   | ND                           |
|             | PE-fortified        | ND                                     | ND                        | ND                           | ND                                  | 0.26 ± 0.05 <sup>A</sup>   | ND                           |
| Day 28      | Control             | ND                                     | 0.07 ± 0.01 <sup>a</sup>  | ND                           | ND                                  | 0.20 ± 0.05 <sup>a</sup>   | ND                           |
|             | PE-fortified        | ND                                     | 0.11 ± 0.05 <sup>A</sup>  | ND                           | ND                                  | 0.16 ± 0.07 <sup>A</sup>   | ND                           |
| Day 56      | Control             | ND                                     | 0.18 ± 0.06 <sup>ab</sup> | 0.12 ± 0.05 <sup>a</sup>     | 0.90 ± 0.19 <sup>a</sup>            | 0.14 ± 0.05 <sup>a</sup>   | ND                           |
|             | PE-fortified        | ND                                     | 0.13 ± 0.09 <sup>A</sup>  | 0.06 ± 0.01 <sup>A</sup>     | ND                                  | 0.27 ± 0.08 <sup>AB*</sup> | ND                           |
| Day 84      | Control             | 0.24 ± 0.20                            | 0.22 ± 0.09 <sup>b</sup>  | 0.34 ± 0.13 <sup>a</sup>     | 2.66 ± 0.45 <sup>b</sup>            | 0.27 ± 0.03 <sup>a</sup>   | 0.22 ± 0.04                  |
|             | PE-fortified        | ND                                     | 0.26 ± 0.08 <sup>B</sup>  | 0.29 ± 0.16 <sup>A</sup>     | 0.89 ± 0.27 <sup>A*</sup>           | 0.41 ± 0.07 <sup>B*</sup>  | 0.37 ± 0.12                  |

Data is presented as mean ± SD (n ≥ 3). When applicable, two tailed unpaired t-test or two-way ANOVA followed by Tukey's or Šidák post hoc test were carried out. Different superscript lower-case letters (a, b) within each column indicate statistically significant difference (p < 0.05) between storage days for the control oils, while different upper-case letters (A, B) show statistically significant difference (p < 0.05) between storage days for the fortified oils. Asterisks (\*) on the values of the fortified oils express a statistically significant difference (p < 0.05) compared to their control counterpart on the same storage day. TG: triacylglycerol; ND: not detected.

**Table 3.** Antioxidant capacities of non-fortified (control) and PE-fortified vegetable oils.

| Oil                | DPPH at 25 °C (%)       |                         | DPPH at 100 °C (%)      |                           | FRAP (μmol Fe(II)/kg)   |                         | Metal chelating potential (%) |                          |
|--------------------|-------------------------|-------------------------|-------------------------|---------------------------|-------------------------|-------------------------|-------------------------------|--------------------------|
|                    | Control                 | PE-fortified            | Control                 | PE-fortified              | Control                 | PE-fortified            | Control                       | PE-fortified             |
| Walnut oil         | 67.9 ± 4.7 <sup>a</sup> | 69.7 ± 4.6 <sup>a</sup> | 79.8 ± 2.7 <sup>a</sup> | 101.7 ± 14.8 <sup>b</sup> | 258 ± 51 <sup>a</sup>   | 293 ± 125 <sup>a</sup>  | 92.8 ± 7.0 <sup>a</sup>       | 85.4 ± 15.0 <sup>a</sup> |
| Safflower seed oil | 35.8 ± 5.0 <sup>a</sup> | 30.4 ± 6.5 <sup>a</sup> | 78.6 ± 2 <sup>a</sup>   | 79.3 ± 3.1 <sup>a</sup>   | 2966 ± 213 <sup>a</sup> | 2844 ± 179 <sup>a</sup> | 105.5 ± 0.8 <sup>a</sup>      | 107.5 ± 0.6 <sup>a</sup> |
| Flaxseed oil       | 59.5 ± 9.1 <sup>a</sup> | 50.0 ± 9.0 <sup>a</sup> | 81.1 ± 5.4 <sup>a</sup> | 77.0 ± 2.4 <sup>a</sup>   | 1666 ± 199 <sup>a</sup> | 1548a ± 60 <sup>a</sup> | 83.8 ± 2.6 <sup>a</sup>       | 86.0 ± 1.4 <sup>a</sup>  |

Data is presented as mean ± SD (n ≥ 4). Different superscript lower-case letters (a, b) show statistically significant difference (p < 0.05) between a fortified oil and its control counterpart, as determined by Welch's t-test or two-way ANOVA followed by Šídák's post hoc test. DPPH: 2,2-diphenyl-1-picrylhydrazyl radical; FRAP: ferric reducing antioxidant power.

### Figure Captions

**Fig. 1.** Oxidation induction time (OIT) at 100 °C for non-fortified (control) and PE-fortified vegetable oils. Data is presented as mean ± SD (n ≥ 3). Different lower-case letters (a, b) show statistically significant difference (p < 0.01) between oils from the same seed, as determined by two-tailed unpaired t-test.

**Fig. 2.** Peroxide value (POV) of walnut (A), safflower seed (B), and flaxseed (C) oils stored under household conditions. For each day of storage, ns indicates no statistically significant difference (p > 0.05) between a fortified oil group and its control counterpart, as determined by unpaired t-tests followed by Holm-Šídák's multiple comparisons corrections.

**Fig. 3.** Concentration of linolenic and/or linoleic acyl groups in walnut (A-B), safflower seed (C), and flaxseed (D-E) oils, as well as their total hydroperoxides (OOHs) content (walnut oil – F, safflower seed oil – G, flaxseed oil – H), after different storage times under household

conditions. Data is presented as mean  $\pm$  SD ( $n \geq 3$ ). Different in-graph lower-case letters (a, b, c, d, e, f) indicate statistically significant difference ( $p < 0.05$ ) between storage days for the control oils, while different upper-case letters (A, B, C, D, E, F) show statistically significant difference ( $p < 0.05$ ) between storage days for the fortified oils. Asterisks (\*) on the fortified oils bars denote a statistically significant difference ( $p < 0.05$ ) in comparison to the respective control on the same storage day. Significant differences were determined by two-way analysis of variance (ANOVA) or Welch's ANOVA, followed by Tukey's or Šídák's post hoc test. TG: triacylglycerol; ND: not detected.

**Fig.4.** Ratios between the peak area of volatile oxidation compounds identified in PE-fortified safflower seed oil and their corresponding peak area in its non-fortified oil counterpart (A), as well as their evolution throughout storage for selected representative cases, namely pentanal (B), hexanal (C), 2-heptenal (D), and 2-octenal (E). Data in A is presented as mean fold-change (FC), whereas data in B-E is presented as mean  $\pm$  SD ( $n \geq 3$ ). Statistically significant differences between fortified and non-fortified control oils are indicated in A with \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ), \*\*\* ( $p < 0.001$ ) and \*\*\*\* ( $p < 0.0001$ ). Conversely, different lower-case letters (a,b) in B-E indicate statistically significant difference ( $p < 0.05$ ) between storage days for the control oils, while different upper-case letters (A, B) show statistically significant difference ( $p < 0.05$ ) between storage days for the fortified oils, as determined by two-way analysis of variance (ANOVA) followed by Tukey's or Šídák's post hoc test. AUC: area under curve.

**Fig. 5.** Concentration of tocopherols in walnut (A, C, E), flaxseed (B, D) and safflower seed (F) oils, determined either by  $^1\text{H}$  NMR (A, B) or HPLC-DAD. Data is presented as mean  $\pm$  SD ( $n \geq 3$ ). Different in-graph lower-case letters (a, b, c) indicate statistically significant difference ( $p < 0.05$ ) between storage days for the control oils, while different in-graph



upper-case letters (A, B, C) show statistically significant difference ( $p < 0.05$ ) between storage days for the fortified oils. Asterisks (\*) on the fortified oils bars denote statistically significant difference ( $p < 0.05$  in comparison to the respective control on the same storage day, as determined by two-way analysis of variance (ANOVA) followed by Tukey's or Šídák's post hoc test. LOD: limit of detection; ND: not detected.

**Fig. 6.** Decimal logarithm of the concentration of 4-hydroxy-2-nonenal (A), 2,4-decadienal (B), and acrolein (C) after heating them at 100 °C for 16 h in the presence or absence of PE(16:0/18:1), as well as the content of 2-octenal (D), nonanal (E), 2-decenal (F), and 2-undecenal (G) in non-fortified and PE-fortified flaxseed oil heated at 100 °C for 10 h. Data is presented as mean  $\pm$  SD ( $n \geq 3$ ). Different lower-case letters (a, b) show statistically significant difference ( $p < 0.05$ ) between non-fortified (control) and PE-fortified groups, as determined by two-tailed unpaired t-test or Welch's t-test. AUC: area under curve; ND: not detected.

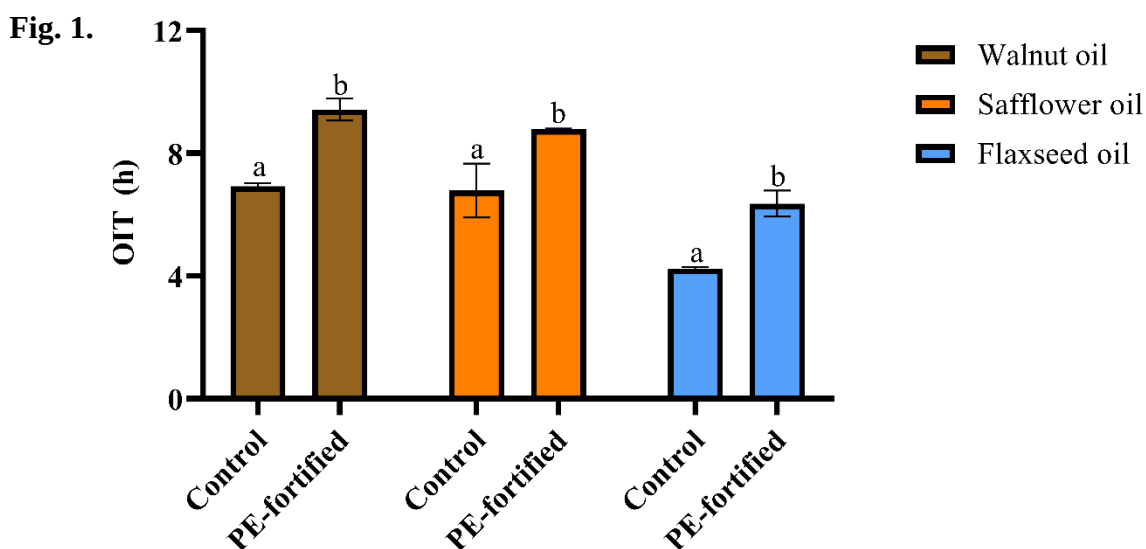


Fig. 2.

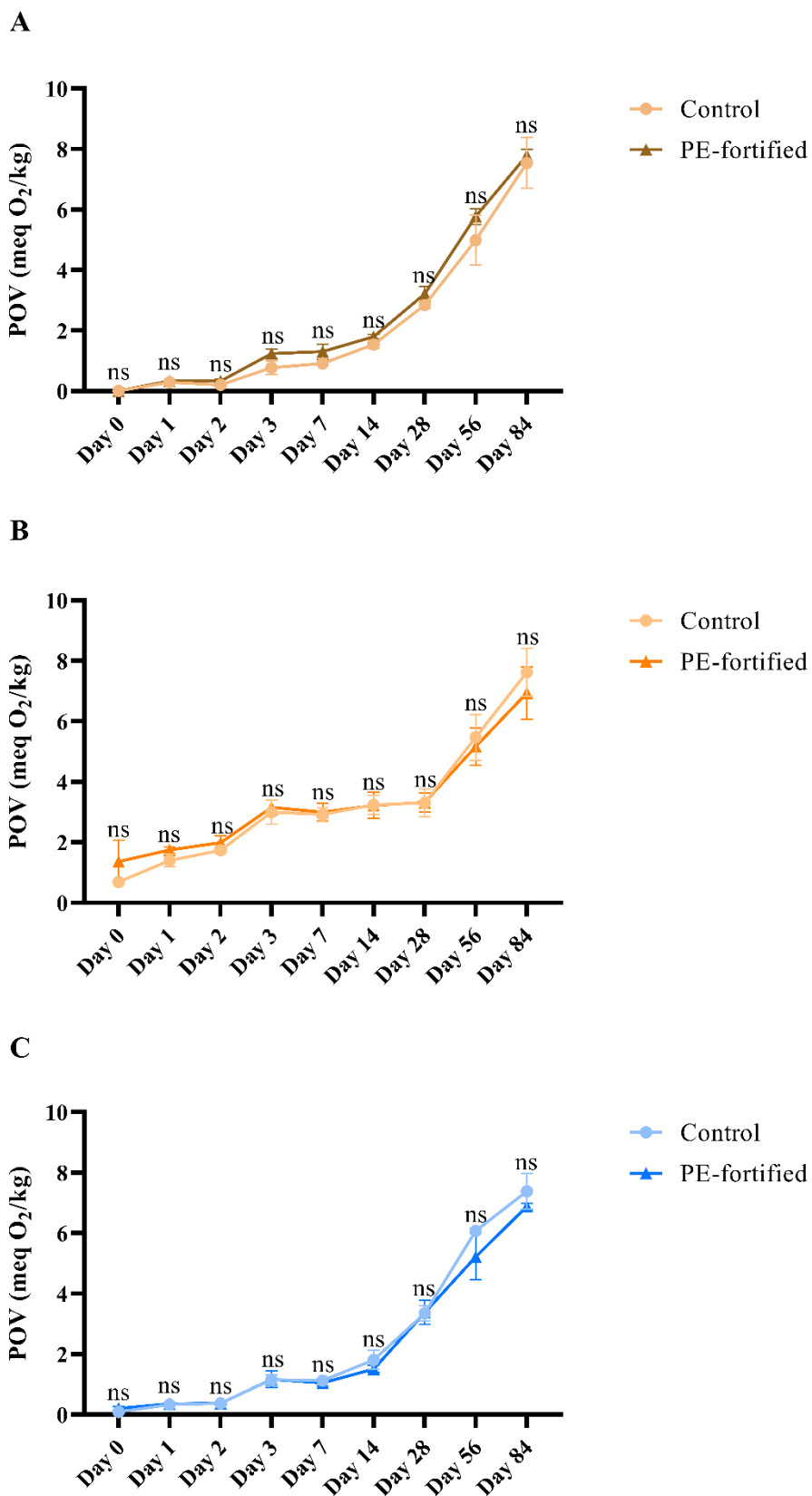


Fig. 3

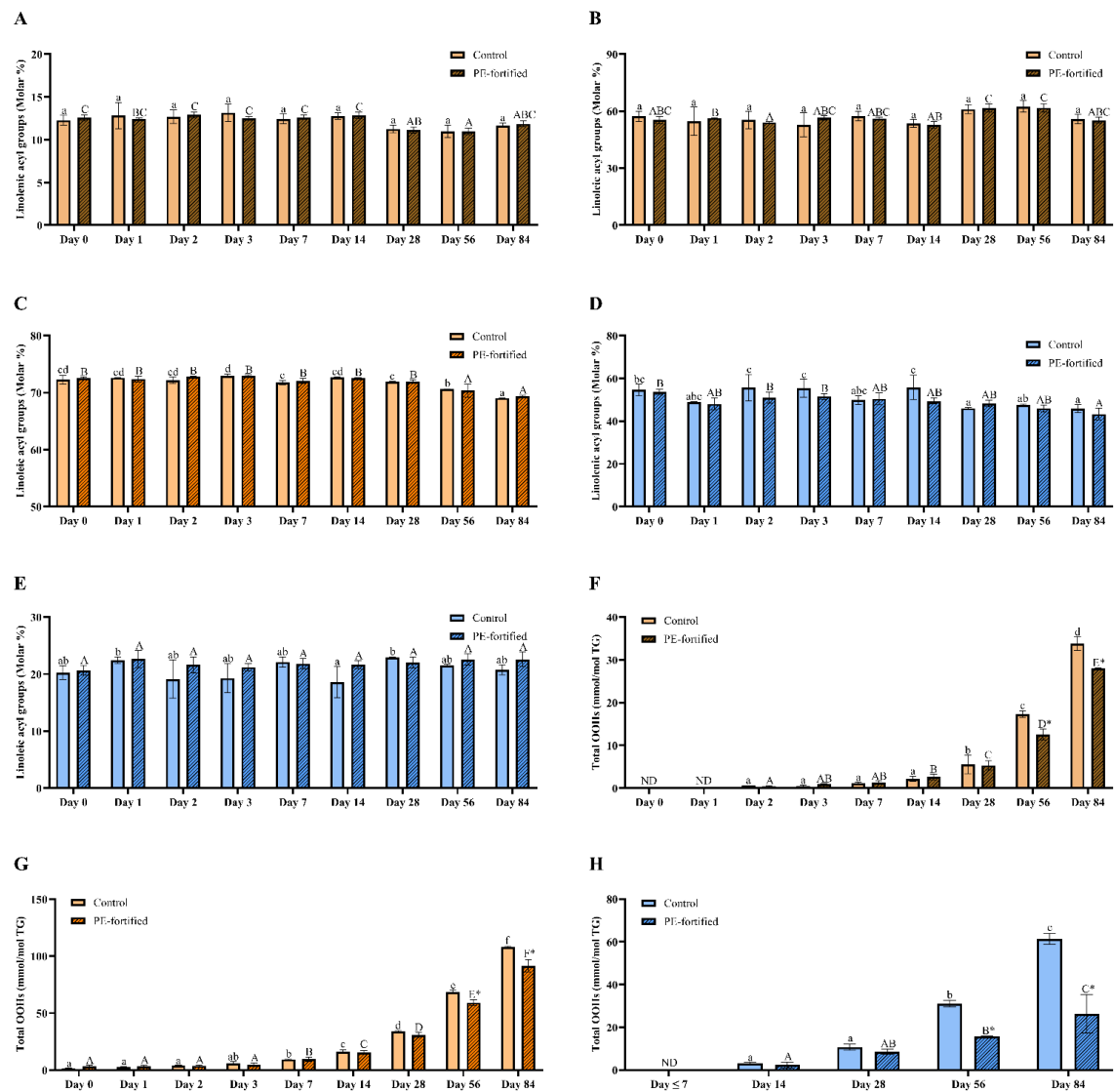
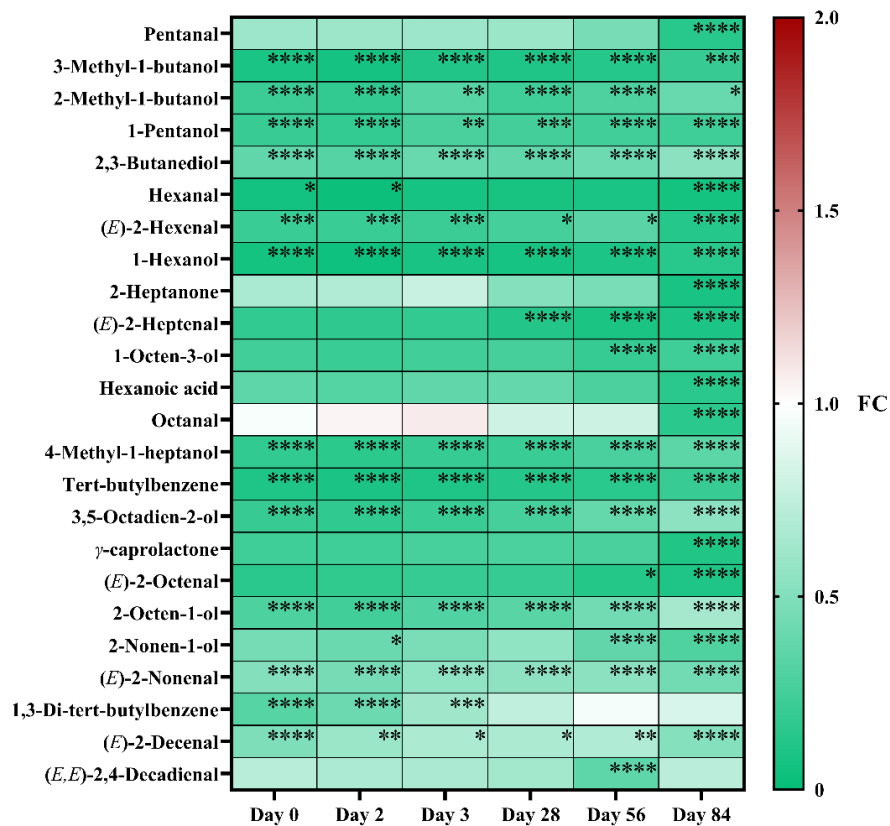
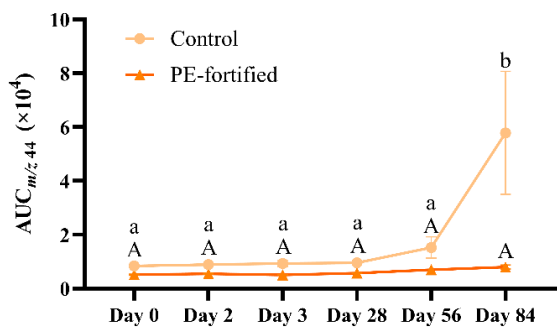


Fig. 4.

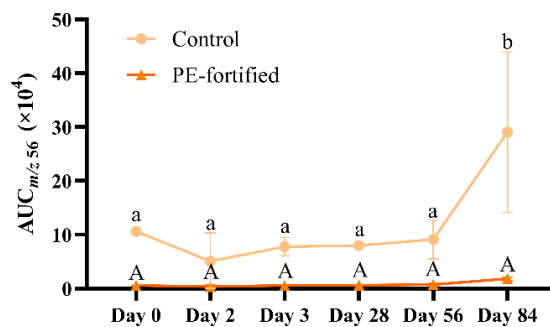
A



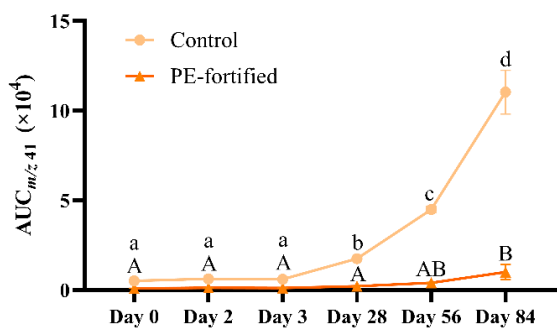
B



C



D



E

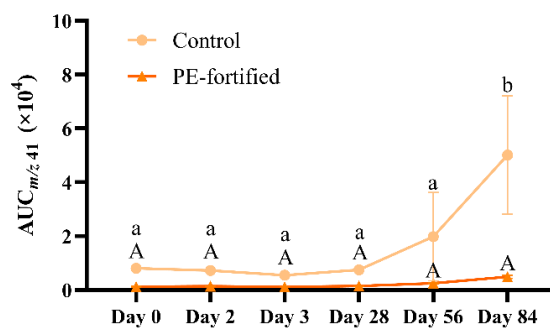
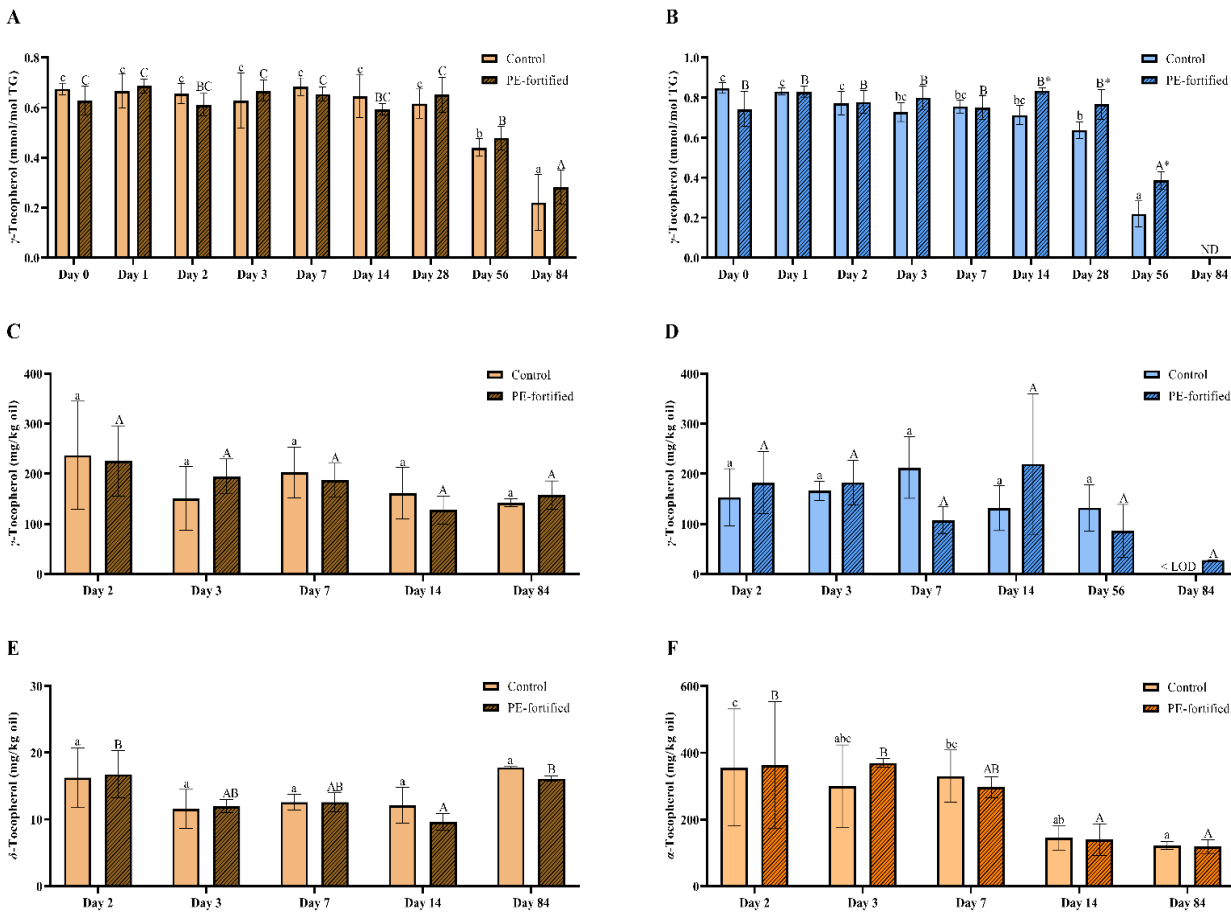
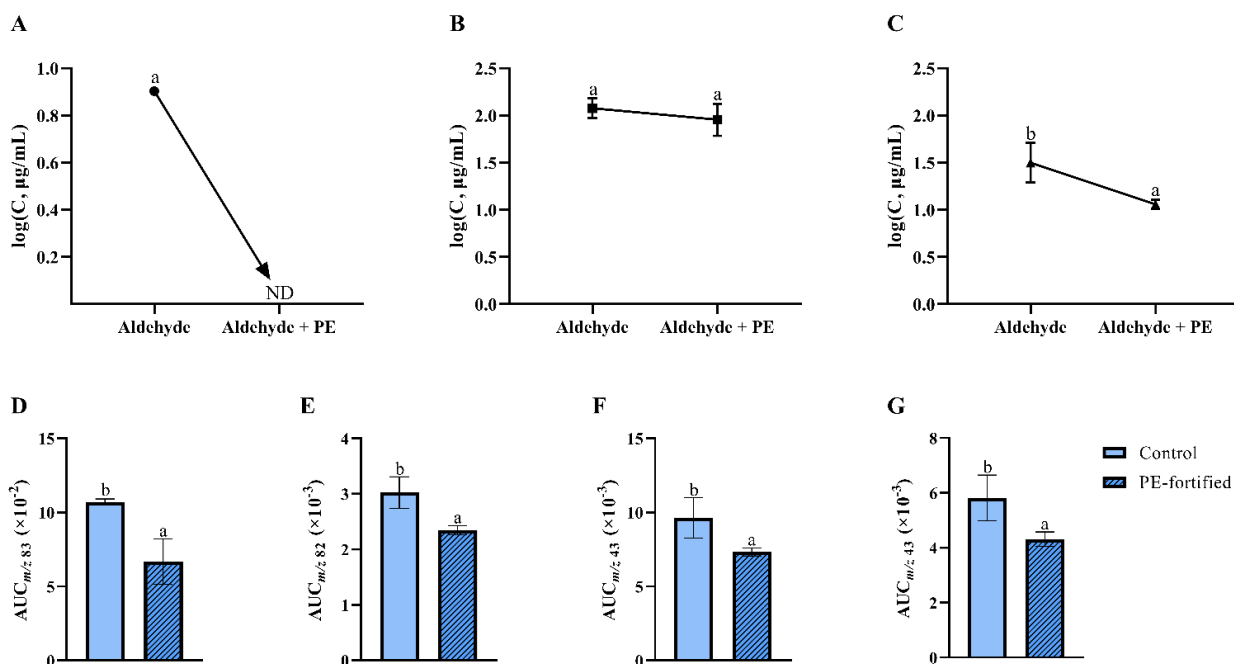


Fig. 5.





**Fig. 6.**

## SUPPLEMENTARY MATERIAL

### Method S1. Reagents and chemicals

Hydrochloric acid, iron(III) chloride, iron(II) chloride, ammonium thiocyanate, ammonium formate,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol,  $\delta$ -tocopherol, toluene, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), sodium acetate trihydrate, and ferrozine were obtained from Sigma-Aldrich (Vienna, Austria). Iron(II) sulphate heptahydrate, barium chloride dihydrate, chloroform, ethanol, n-hexane, acetic acid, and MS-grade 2-propanol were all purchased from Carl Roth (Karlsruhe, Germany), while deuterated chloroform, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and rac-tocol were obtained from Eurisotop (Tewksbury, MA, USA), Iris Biotech (Marktredwitz, Germany), and Cayman Chemicals (Ann Arbor, MI, USA), respectively. Lastly, MS-grade water, methanol, acetonitrile, and formic acid, as well as hydrogen peroxide, were acquired from Avantor/VWR International (Vienna, Austria).

### Method S2. Determination of peroxide value (POV) by International Dairy Federation (IDF) assay

POV was determined in the oil samples using the IDF method (International Dairy Federation,

1991). As described by Shantha and Decker (1994), an iron(II) chloride solution was obtained by gradually mixing 50 mL 8 g/L barium chloride dihydrate, 50 mL 10 g/L iron(II) sulfate heptahydrate, and 2 mL 10 mol/L hydrochloric acid, followed by filtration on filter paper. A total of 15-25 mg oil was then dissolved in 9.9 mL chloroform:methanol 7:3 mix (v/v) and 50  $\mu$ L ammonium 0.3 g/mL thiocyanate, and 50  $\mu$ L of the prepared iron(II) chloride solution were subsequently added. After a 5 min incubation at room temperature, samples were transferred to quartz cuvettes and their absorbance at 500 nm was immediately registered with a Spark® Tecan spectrophotometer (Männedorf, Switzerland). In addition, a calibration curve using different concentrations of an iron(III) chloride solution instead of oil was measured (Table S1). The POV of the samples was calculated as in Eq. S1:

$$\text{meq O}_2/\text{kg sample} = (\text{Abs}_{500\text{S}} - \text{Abs}_{500\text{B}}) / (k \cdot 55.84 \cdot E \cdot 2) \quad (\text{Eq. S1})$$

where Abs500S and Abs500B are the absorbances at 500 nm of samples and blanks, respectively; k is the slope of the calibration curve, 55.84 is the atomic mass of iron (g/mol), E is the initial weight of the sample (g), and 2 is the conversion factor to peroxide milliequivalents (meq), which corresponds to 2 mmol in SI units. Results are expressed in peroxide milliequivalents (meq) per kg oil, which corresponds to 2 mmol O<sub>2</sub>/kg in SI units.

**Method S3.** Analysis of volatile oxidation compounds by solid phase microextraction followed by gas chromatography-mass spectrometry (SPME/GC-MS)

The content of volatile oxidation products in the safflower seed oil samples was evaluated as described by Alberdi-Cedeño, Ibargoitia, and Guillén (2020), with modifications. After weighing  $2.50 \pm 0.02$  g oil in a 20 mL headspace brown glass vial and a 5 min equilibration period, volatile compounds were extracted via SPME using a fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (50/30  $\mu$ m film thickness, 1 cm length) for 55 minutes at 50 °C, and injected into a GC-QP2010 system Ultra (Shimadzu, Korneuburg, Austria), with desorption time 10 min and purge time 15 min. Volatile compounds were separated in a Zebron ZB-5 ms column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m, Phenomenex, Aschaffenburg, Germany), with the oven temperature gradient starting at 50 °C for 5 min and subsequently increasing to 300 °C at a rate of 5 °C/min, followed by a hold step at 300 °C for 17 min. Helium was used as carrier gas at a constant pressure of 117 kPa, and the injector and the interface were set at 205 °C and 305 °C, respectively. The temperature was 230 °C for the ion source and 150 °C for the quadrupole mass analyzer, with ionization energy 70 eV and mass range m/z 41-600. Identification of volatile compounds was carried out via matching the experimental mass spectra

with a commercial library (NIST, ver. 11.0), with an identity score equal or higher to 85%. For comparative purposes between samples, semi-quantification was done by obtaining the area under the curve for one the main ion peaks of each compound (Table S2).

**Method S4.** Determination of tocopherols by high performance liquid chromatography-diode array detection (HPLC-DAD)

The content of vitamin E in the oil samples from selected storage days was analyzed as outlined by Pignitter et al. (2014). Briefly, 50 mg oil were dissolved in 2-propanol and 1  $\mu$ L 5 mg/mL rac-tocol was added as internal standard. After filtration through 0.22  $\mu$ m polyvinylidene fluoride (PVDF) syringe filters into HPLC vials, 40  $\mu$ L sample were injected into a Shimadzu LC-20AD system coupled to a Nexera X2 SPD-M30A diode array detector. Using water and methanol as mobile phases A and B, respectively, tocopherols were separated on a Shim-pack VP-ODS column (150 mm  $\times$  4.6 mm  $\times$  5  $\mu$ m) equipped with a Shim-pack GVP-ODS precolumn (10 mm  $\times$  4.6 mm  $\times$  5  $\mu$ m), with the following gradient: 0-4 min 95-100% B; 4-34 min 100% B; 34-36 min 100-95% B; 36-38 min 95% B. The oven temperature was set to 10  $^{\circ}$ C and the flow rate was 0.5 mL/min. Detection wavelength was 295 nm. Data was normalized to the rac-tocol area, prior to quantification of  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocopherol via external standard curves (Table S1).

**Method S5.** Analysis of free radical scavenging activity by diphenyl-1-picrylhydrazyl radical (DPPH) assay

The radical scavenging activity of freshly prepared non-fortified and fortified oil samples was assessed as described by Goritschnig et al. (2020). In short, 0.25  $\mu$ L oil sample were added to 1 mL 0.25 mmol/L DPPH in toluene and incubated in the dark at 25  $^{\circ}$ C for 1 h. Afterwards, 100  $\mu$ L each sample were transferred to a 96-well clear plate, to measure their absorbance at 520 nm with a Spark<sup>®</sup> Tecan spectrophotometer. Results were expressed as the percentage of remaining DPPH in relation to the absorbance of blanks without oil. To test the free radical scavenging activity of PE(16:0/18:1) under heating conditions, the experiment was repeated with 100  $^{\circ}$ C as the incubation temperature.

**Method S6.** Analysis of ferric ion reducing power (FRAP)

Phenolic extracts were first obtained from freshly prepared non-fortified and fortified oil samples according to Pointner et al. (2024). The FRAP assay described by Benzie & Strain (1996) was then employed as follows: a total of 25  $\mu$ L from these extracts were dissolved in 1 mL FRAP working solution obtained by mixing 5 mL 3.3 mg/mL TPTZ in 40 mmol/L hydrochloric acid, 5

mL 20 mmol/L iron(II) chloride, and 50 mL FRAP buffer, which contained 150 mg sodium acetate trihydrate and 800  $\mu$ L acetic acid. The FRAP working solution was freshly prepared and warmed up to 37 °C for 30 min prior to use. Once the samples were dissolved, their content was transferred to a cuvette and their absorbance at 593 nm was measured after 6 min. Results were expressed in  $\mu$ mol iron(II)/L, in relation to an iron (II) sulphate heptahydrate standard curve (Table S1).

#### Method S7. Statistical analysis

Normal distribution of the results was evaluated by Shapiro-Wilk test, significant outliers ( $p < 0.05$ ) were excluded according to Grubb's test, and equal variances between groups were evaluated by Brown-Forsythe test. When the homogeneity of variances condition was met, statistically significant differences ( $p < 0.05$ ) between groups were evaluated by two-tailed unpaired t-test or one- or two-way analysis of variance (ANOVA) followed by Tukey's or Šídák's post hoc test, depending on the groups compared. In case of heterogeneity of variances, Welch's t-test or Welch's ANOVA followed by Dunett's T3 test were used. Correlation analyses were carried out via two-tailed Pearson's correlation test. For quantitative determinations, limits of detection (LOD) and quantification (LOQ) were determined based on a ratio of 3.3 and 10 between the residual standard deviation of the curves and their slopes, respectively (ICH Harmonised Tripartite Guideline, 2005).

**Table S1.** Representative standard curves and quantification parameters used in this study.

| Analysis                | Curve equation                | R <sup>2</sup> | Range                   | LOD             | LOQ             |
|-------------------------|-------------------------------|----------------|-------------------------|-----------------|-----------------|
| Peroxide value          | $y = 0.1061 \cdot x + 0.0005$ | 0.9992         | 0.5 – 4.0 $\mu$ g/mL    | 0.1 $\mu$ g/mL  | 0.3 $\mu$ g/mL  |
| $\alpha$ -Tocopherol    | $y = 21688 \cdot x - 7963$    | 0.9995         | 5.0 – 100.0 $\mu$ g/mL  | 1.4 $\mu$ g/mL  | 4.3 $\mu$ g/mL  |
| $\gamma$ -Tocopherol    | $y = 27788 \cdot x + 7059$    | 0.9997         | 5.0 – 100.0 $\mu$ g/mL  | 1.0 $\mu$ g/mL  | 2.9 $\mu$ g/mL  |
| $\delta$ -Tocopherol    | $y = 32521 \cdot x - 10953$   | 0.9996         | 5.0 – 100.0 $\mu$ g/mL  | 1.1 $\mu$ g/mL  | 3.5 $\mu$ g/mL  |
| 4-hydroxy-(E)-2-nonenal | $y = 0.0008 \cdot x - 0.0009$ | 0.9840         | 6.0 – 10.0 $\mu$ g/mL   | 1.6 $\mu$ g/mL  | 4.8 $\mu$ g/mL  |
| (E,E)-2,4-decadienal    | $y = 0.0004 \cdot x + 0.0006$ | 0.9882         | 51.0 – 102.0 $\mu$ g/mL | 15.0 $\mu$ g/mL | 45.6 $\mu$ g/mL |
| Acrolein                | $y = 0.0004 \cdot x - 0.0020$ | 0.9975         | 20.0 – 35.0 $\mu$ g/mL  | 2.7 $\mu$ g/mL  | 8.3 $\mu$ g/mL  |

LOD: Limit of detection; LOQ: Limit of quantification.

**Table S2.** Volatile lipid oxidation compounds detected by GC-MS

| Volatile compound                | Retention time<br>(min) | Fragment ion<br>( <i>m/z</i> ) |
|----------------------------------|-------------------------|--------------------------------|
| Pentanal                         | 2.6                     | 44                             |
| 3-Methylbutanal                  | 2.7                     | 58                             |
| 3-Methyl-1-butanol               | 3.1                     | 55                             |
| 2-Methyl-1-butanol               | 3.2                     | 41                             |
| 1-Pentanol                       | 3.6                     | 42                             |
| 2,3-Butanediol                   | 4.0                     | 45                             |
| Hexanal                          | 4.6                     | 56                             |
| ( <i>E</i> )-2-Hexenal           | 6.0                     | 41                             |
| 1-Hexanol                        | 6.7                     | 56                             |
| 2-Heptanone                      | 7.4                     | 43                             |
| Heptanal                         | 7.9                     | 70                             |
| ( <i>E</i> )-2-Heptenal          | 9.9                     | 41                             |
| 5-Ethyl-2(5H)-furanone           | 10.0                    | 83                             |
| ( <i>E,E</i> )-2,4-Heptadienal   | 11.1                    | 81                             |
| Hexanoic acid                    | 11.3                    | 60                             |
| Octanal                          | 11.6                    | 43                             |
| 2-Octen-1-ol                     | 13.1                    | 55                             |
| 4-Methyl-1-heptanol              | 12.0                    | 69                             |
| Tert-butylbenzene                | 12.1                    | 119                            |
| 3,5-Octadien-2-ol                | 12.6                    | 55                             |
| $\gamma$ -caprolactone           | 13.0                    | 85                             |
| ( <i>E</i> )-2-Octenal           | 13.5                    | 41                             |
| 1-Octen-3-ol                     | 13.6                    | 57                             |
| 3,5-Dimethylcyclohexanol         | 14.7                    | 71                             |
| Nonanal                          | 15.0                    | 82                             |
| 2-Nonen-1-ol                     | 15.4                    | 57                             |
| ( <i>E</i> )-2-Nonenal           | 16.8                    | 55                             |
| Decanal                          | 18.2                    | 43                             |
| 1,3-Di-tert-butylbenzene         | 19.3                    | 175                            |
| ( <i>E</i> )-2-Decenal           | 19.9                    | 43                             |
| Nonanoic acid                    | 20.0                    | 60                             |
| ( <i>E,E</i> )-2,4-Decadienal    | 21.5                    | 81                             |
| ( <i>E</i> )-2-Undecenal         | 22.8                    | 43                             |
| 4,5-Epoxy-( <i>E</i> )-2-decenal | 23.1                    | 68                             |
| Pentanal                         | 2.6                     | 44                             |
| 3-Methylbutanal                  | 2.7                     | 58                             |
| 3-Methyl-1-butanol               | 3.1                     | 55                             |



| Volatile compound                    | Retention time<br>(min) | Fragment<br>ion<br>( <i>m/z</i> ) |
|--------------------------------------|-------------------------|-----------------------------------|
| 2-Methyl-1-butanol                   | 3.2                     | 41                                |
| 1-Pentanol                           | 3.6                     | 42                                |
| 2,3-Butanediol                       | 4.0                     | 45                                |
| Hexanal                              | 4.6                     | 56                                |
| ( <i>E</i> )-2-Hexenal               | 6.0                     | 41                                |
| 1-Hexanol                            | 6.7                     | 56                                |
| 2-Heptanone                          | 7.4                     | 43                                |
| Heptanal                             | 7.9                     | 70                                |
| ( <i>E</i> )-2-Heptenal              | 9.9                     | 41                                |
| 5-Ethyl-2(5H)-<br>furanone           | 10.0                    | 83                                |
| ( <i>E,E</i> )-2,4-Heptadienal       | 11.1                    | 81                                |
| Hexanoic acid                        | 11.3                    | 60                                |
| Octanal                              | 11.6                    | 43                                |
| 2-Octen-1-ol                         | 13.1                    | 55                                |
| 4-Methyl-1-heptanol                  | 12.0                    | 69                                |
| Tert-butylbenzene                    | 12.1                    | 119                               |
| 3,5-Octadien-2-ol                    | 12.6                    | 55                                |
| $\gamma$ -caprolactone               | 13.0                    | 85                                |
| ( <i>E</i> )-2-Octenal               | 13.5                    | 41                                |
| 1-Octen-3-ol                         | 13.6                    | 57                                |
| 3,5-Dimethylcyclohexanol             | 14.7                    | 71                                |
| Nonanal                              | 15.0                    | 82                                |
| 2-Nonen-1-ol                         | 15.4                    | 57                                |
| ( <i>E</i> )-2-Nonenal               | 16.8                    | 55                                |
| Decanal                              | 18.2                    | 43                                |
| 1,3-Di-tert-<br>butylbenzene         | 19.3                    | 175                               |
| ( <i>E</i> )-2-Decenal               | 19.9                    | 43                                |
| Nonanoic acid                        | 20.0                    | 60                                |
| ( <i>E,E</i> )-2,4-Decadienal        | 21.5                    | 81                                |
| ( <i>E</i> )-2-Undecenal             | 22.8                    | 43                                |
| 4,5-Epoxy-( <i>E</i> )-2-<br>decenal | 23.1                    | 68                                |

Data is presented as mean  $\pm$  SD ( $n \geq 3$ ). Different lower-case letters (a, b) for the same compound indicate statistically significant difference ( $p < 0.05$ ) due to PE fortification, as determined by two-tailed unpaired t-test or Welch's t-test. AUC: area under curve.

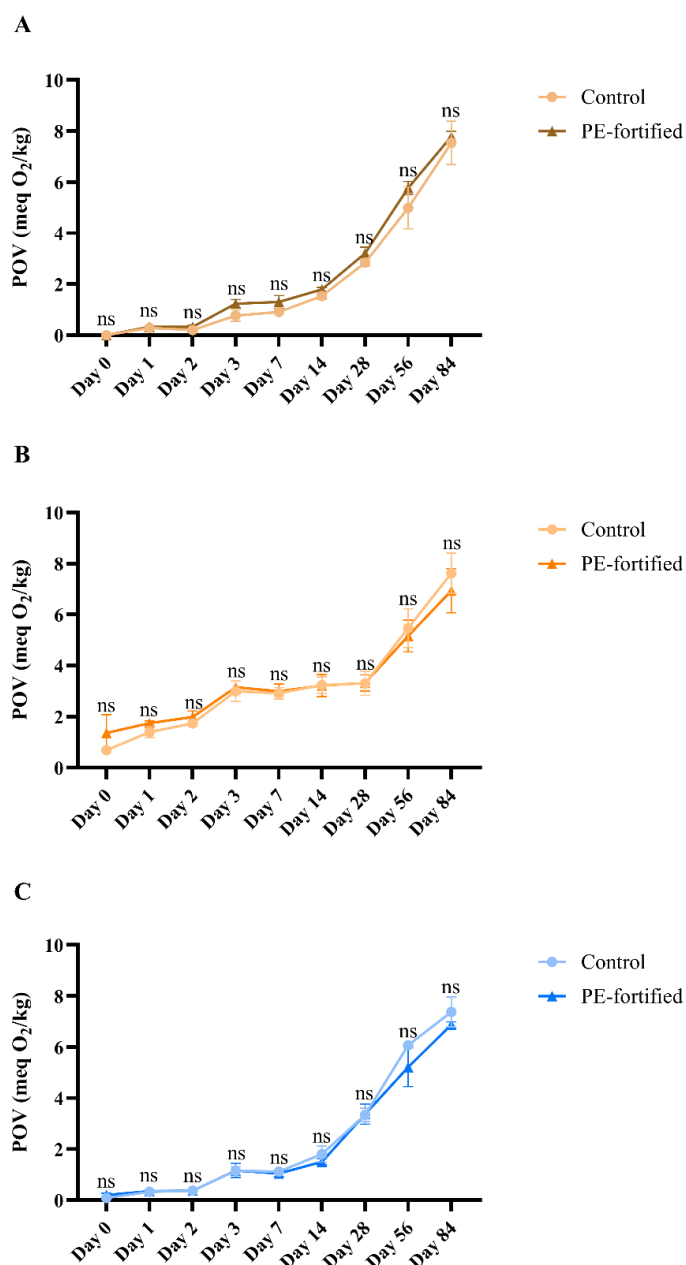


Fig. S1. Peroxide value (POV) of walnut (A), safflower seed (B), and flaxseed (C) oils stored under household conditions. For each day of storage, ns indicates no statistically significant difference ( $p > 0.05$ ) between a fortified oil group and its control counterpart, as determined by unpaired t-tests followed by Holm-Šidák's multiple comparisons corrections.



Fig. S2. Non-fortified (left) and PE-fortified (right) flaxseed oil after heating at 100°C for 10 h.

## References

- Alberdi-Cedeño, J., Ibargoitia, M. L., & Guillén, M. D. (2020). A global study by <sup>1</sup>H 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(4), Article 312. <https://doi.org/10.3390/antiox9040312>.
- 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(1), 70-76. <https://doi.org/10.1006/abio.1996.0292>.
- Goritschnig, J., Tadás, K., König, J., & Pignitter, M. (2020). Free radical scavenging activity of carbonyl-amine adducts formed in soybean oil fortified with phosphatidylethanolamine. *Molecules*, 25(2), Article 373. <https://doi.org/10.3390/molecules25020373>.
- ICH Harmonised Tripartite Guideline. (2005). Validation of analytical procedures: text and methodology.
- International Dairy Federation. (1991). Standard 74A.1991.
- Anhydrous milkfat: determination of peroxide value. Square Vergote 41, Brussels, Belgium
- 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(10), 2297-2305. <https://doi.org/10.1021/jf405736j>.
- Pointner, T., Rauh, K., Auñón-Lopez, A., Kostadinović Veličkovska, S., Mitrev, S., Arsov, E., & Pignitter, M. (2024). Comprehensive analysis of oxidative stability and nutritional values of germinated linseed and sunflower seed oil. *Food Chemistry*, 454, Article 139790. <https://doi.org/10.1016/j.foodchem.2024.139790>.
- Shantha, N. C., & Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC INTERNATIONAL*, 77(2), 421-424. <https://doi.org/10.1093/jaoac/77.2.421>.

## 5 PART III: SYSTEMATIC BIOMARKER IDENTIFICATION

### 5.1 RESEARCH SCOPE

Part III of this thesis focused on developing an analytical and data-driven approach to identify predictive biomarkers of oxidative degradation in cold-pressed oils. This study aimed to understand how and why deterioration occurs, by systematically characterizing chemical changes over time and further translating them into measurable indicators of shelf-life. Through a multi-marker ML approach, this part established a comprehensive model linking compositional dynamics with oxidative stability under realistic storage conditions.

The study monitored six cold-pressed oils (black cumin, canola, sunflower, high-oleic sunflower, linseed and hempseed oil) over a period of six months under household conditions. The three complementary parameters of fatty acid profile (GC-FID), polyphenol degradation (LC-MS/MS) and secondary lipid oxidation product formation (SPME-GC-MS) were tracked over time. Together, these datasets captured the progression from primary oxidation to advanced volatile markers, as well as the depletion of antioxidants.

To extract robust predictive information from this complex dataset, a biomarker identification pipeline was established based on Least Absolute Shrinkage and Selection Operator (LASSO), Random Forest regression (RFR) and correlation analysis. These models were applied to rank and weight the most time-responsive compounds across oils, generating oil-specific degradation fingerprints. Through this integrative approach oil-type-specific biomarkers were identified. For instance, linoleic and  $\alpha$ -linolenic acid were ranked as main oxidation markers and compounds such as hexanal, 2,4-decadienal or p-coumaric acid as distinct deterioration markers in specific oils.

By merging targeted analytical data sets with multivariate modeling, Part III provides a quantitative foundation for predicting shelf-life and oxidative stability of cold-pressed oils. The results demonstrate that oil quality deterioration cannot be captured by a single marker but instead requires a multi-compound approach reflecting the complex interplay of fatty acid degradation, antioxidant consumption and volatile accumulation. Ultimately, this part establishes a methodological basis for future rapid detection tools, such as e-nose or NIR spectroscopy models, enabling real-time quality assessment of cold-pressed oils.



## 5.2 Original Works

### 5.2.1 Fatty Acids, Polyphenols and Volatiles as Predictive Biomarkers of Cold-Pressed Oil Stability

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**Keywords:** Cold-pressed oils, Oxidative stability, Biomarker identification, Fatty acids, Polyphenols, Secondary lipid oxidation products

**Status:** Submitted (to Food Chemistry: X)

**Contribution:** I designed and conceptualized the study, developed the analytical workflow and data integration strategy and carried out the experimental work, including GC-FID, LC-MS/MS, and GC-MS analyses. I performed data processing, coding and biomarker modeling, statistical evaluation and prepared figures. I wrote the original manuscript draft and contributed to the interpretation and presentation of the results.

## Abstract

Cold-pressed vegetable oils are rich in PUFAs and bioactive polyphenols but are highly susceptible to oxidative deterioration. Six cold-pressed oils (black cumin, canola, sunflower, high-oleic sunflower, linseed and hempseed oil) from three Austrian suppliers each were stored under household-relevant conditions over six months and analyzed by GC-FID, LC-MS/MS and SPME-GC-MS. Biomarker identification employed Least Absolute Shrinkage and Selection Operator (LASSO), Random Forest regression (RFR) and correlation analysis. Fatty acid degradation, particularly of linoleic and  $\alpha$ -linolenic acid, was the main driver of oxidation, while volatile aldehydes such as hexanal, 2,4-decadienal, and (E)-2-nonenal served as key secondary markers. Black cumin oil exhibited rapid loss of p-coumaric acid (−87% after 14 days) despite its high initial phenolic content ( $745 \pm 227 \mu\text{g/g}$ ). This is the first study to systematically identify oil-type-specific deterioration biomarkers using an integrated, machine-learning-based multi-marker approach, demonstrating that oxidative stability in cold-pressed oils is best characterized by multi-markers.

## 1. Introduction

Vegetable oils are increasingly recognized for their nutritional value due to their rich content of mono- and polyunsaturated fatty acids (MUFAs and PUFAs) (Koch et al., 2023). Their dietary consumption has been associated with various health benefits. Particularly, oils rich in PUFAs have been shown to positively influence blood pressure regulation and reduce the risk of coronary heart disease (Haque et al., 2024). However, adverse health effects such as hepatic inflammation may arise from the consumption of lipid oxidation products formed during processing or storage. Preventing oxidative degradation is therefore essential to ensure oil

quality and safety (Böhm et al., 2013; Kanner, 2007).

Edible oils deteriorate via the two primary oxidative pathways, photooxidation, initiated by ultraviolet and visible light, and autoxidation due to oxygen exposure during storage (Pignitter et al., 2014; Tawfik & Huyghebaert, 1999). These reactions result in the breakdown of unsaturated fatty acids and the formation of volatile compounds associated with rancidity. To reduce the formation of these degradation products, it is recommended to store oils under minimal light and oxygen exposure, at lower temperatures, and in the absence of pro-oxidative metals (Choe & Min, 2006). Additionally, antioxidants such as tocopherols and polyphenols can be added or retained through minimal processing to suppress oxidation (Mehany et al., 2025). Cold-pressed oils, while often richer in bioactive compounds, also contain high levels of PUFAs and are thus particularly prone to autoxidation, posing a major concern for both consumers and producers (Gharby et al., 2025). Secondary lipid oxidation products such as aldehydes and ketones are largely responsible for the characteristic off-flavors and off-odors of rancid oils, which negatively impact sensory quality (Geng et al., 2023). As a result, the shelf-life of edible oils is a critical factor affecting their quality, nutritional value, and marketability (Grajzer et al., 2020).

Despite advances in analytical techniques predicting the oxidative stability and shelf-life of oils remains challenging due to the complex interplay of chemical, physical, and environmental factors (Karami et al., 2020). A number of storage studies have been conducted to assess oil deterioration, primarily by tracking the formation of primary and secondary oxidation products under accelerated conditions (Cui et al., 2024; Holler et al., 2023; Pignitter et al., 2014; Tanouti et al., 2012). Among the most widely studied markers of oil aging are aldehydes such as hexanal, pentanal, and nonanal, which are derived from the oxidation of unsaturated fatty acids and serve as established indicators of rancidity (Parisi, 2016). Hexanal, in particular, has been

proposed as a universal marker due to its sensitivity and prevalence in PUFA oxidation (Jacobsen et al., 2008). However, its reliability as a sole marker has been questioned, as sensory rejection and hexanal levels do not always correlate well, especially across diverse oil matrices. For instance, Ampofo et al. (2022) showed that walnut oil had strong correlation between hexanal and rancidity at low temperatures ( $r \approx 0.92$ ), but at ambient storage (23°C), rancidity perception was associated with multiple other volatiles, reducing hexanal's predictive power. Tocopherols have also been investigated as early depletion markers, with their degradation kinetics suggested as predictive indicators of shelf-life (Bayram et al., 2024). In contrast, other antioxidant compounds such as polyphenols have rarely been studied in detail as biomarkers of oil stability, despite their established role in modulating lipid oxidation. This gap underscores the need for oil-specific degradation profiling that considers not only individual markers, but also the interplay of antioxidants and oxidation products over time.

While many studies focused on either fatty acid degradation, polyphenol depletion, or volatile accumulation in isolation, only few have integrated these chemical classes across extended storage to capture full deterioration pathways. This integration is critical to identify robust biomarkers that reflect both oxidative susceptibility and resistance. To address this, the present study employed a comprehensive analytical approach combining GC-FID for fatty acid profiling, LC-MS/MS for polyphenol quantification and GC-MS for monitoring secondary lipid oxidation products. The aim was to identify robust and oil-type-specific markers for oxidative deterioration in six commonly consumed cold-pressed oils under household conditions over a period of six months. To our knowledge, no previous study has combined multivariate machine learning (ML) approaches with multi-class chemical profiling to derive oil-type-specific deterioration biomarkers. To systematically assess the predictive value of each compound, we applied Least Absolute Shrinkage and Selection Operator (LASSO) and Random Forest

regression (RFR). These approaches are widely recognized for their ability to perform feature selection in high-dimensional omics and food quality datasets (Li et al., 2024; W. Zhang et al., 2025). By integrating these tools with analytical data, we aim to deepen our understanding of lipid oxidation pathways and support the development of future detection strategies.

## 2. Materials and Methods

### 2.1. Chemicals and Materials

All solvents and reagents were of analytical or LC–MS grade. They were obtained from established suppliers including Carl Roth GmbH (Karlsruhe, Germany), Sigma-Aldrich (Vienna, Austria) and VWR International GmbH (Vienna, Austria).

### 2.2. Oil Samples and Conditions of Storage Study

Six cold-pressed vegetable oils were selected based on their diversity, fatty acid composition and commercial relevance: black cumin (BC), canola (CA), sunflower (SF), high-oleic sunflower (HOSF), linseed (LS) and hempseed oil (HS). Each oil type was sourced from three independent Austrian producers to account for batch variability: Schalk Mühle, Weber Michl, and Hagenthaler Ölmanufaktur. A total of 18 oils, with three replicates each, were included in the storage study.

To simulate typical consumer storage household conditions, the oil samples were stored at ambient temperature ( $22 \pm 2^\circ\text{C}$ ) for six months (168 days) in transparent PET bottles. Each oil was stored in triplicate 400 mL volumes, resulting in a total of 54 PET bottles. Samples were exposed to a 12 h light/12 h dark cycle using cold fluorescent lighting ( $\sim 1000$  lx), mimicking household conditions, as it can be seen in Fig S.1. Sampling was conducted at 10 time points (T0-T9 at day 0, 3, 7, 14, 28, 42, 56, 84, 112 and 168). At each of the pre-defined time points 15 mL of oil was removed per bottle and stored in 15 mL polypropylene tubes at  $-80^\circ\text{C}$  until



analysis. This setup allowed tracking of lipid oxidation and antioxidant degradation kinetics in realistic settings over a 6-month period.

### **2.3. Fatty Acid Analysis by GC-FID**

The fatty acid composition of oils was determined as fatty acid methyl esters (FAMES) using gas chromatography with flame ionization detection (GC-FID). Sample preparation followed the protocol from Lall et al. (2009) with modifications by Pignitter et al. (2014) and Grüneis et al. (2019) (Grüneis et al., 2019; Lall et al., 2009; Pignitter et al., 2014). Briefly, 100 mg oil was weighed into a 50 mL tube and spiked with 500  $\mu$ L of a 1% heptadecanoic acid methyl ester (HME) internal standard. Derivatization was carried out with 2 mL toluene, 100 mg pyrogallol, and 4 mL sodium methoxide (0.5 M in methanol), incubated at 50°C for 10 min under argon, and neutralized with 200  $\mu$ L glacial acetic acid. Extraction was performed with 5 mL n-hexane and 5 mL H<sub>2</sub>O, and the organic phase was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and filtered (0.45  $\mu$ m PVDF).

Analyses were performed on a Shimadzu GC-2010 Plus system equipped with an AOC-20i autosampler and a ZB-WAX column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m). Injection volume was 1  $\mu$ L (split 1:20) at 200°C, with helium as carrier gas (3 mL/min). The oven program was 60°C (2 min), ramp 13°C/min to 150°C, then 2°C/min to 240°C. The FID was operated with N<sub>2</sub> (30 mL/min), H<sub>2</sub> (40 mL/min), and air (400 mL/min). Samples were run in triplicate. Calibration was performed with external FAME standards shown in Table S1, and additional FAMES were identified by retention index comparison with literature. Limits of detection (LOD) and quantification (LOQ) were calculated from calibration curve standard deviations.

### **2.4. Polyphenol Profiling by LC-MS/MS**

Polyphenols were extracted according to a modified protocol of Singleton et al. (1965) (Singleton & Rossi, 1965). In brief, 3.5 g oil was extracted with 3.5 mL n-hexane and 1 mL

methanol:H<sub>2</sub>O (80:20, v/v), vortexed (4 min), and centrifuged (6 min, 4°C, 2280 g). The methanol phase was collected and pooled over three sequential extractions. After n-hexane washing, the combined methanol fractions were dried under nitrogen, freeze-dried, and reconstituted in 500 µL methanol (80%).

Polyphenols were analyzed based on Pointner et al. (2024) (Pointner et al., 2024) on a Shimadzu LCMS-8040 triple quadrupole system using a Kinetex C18 column (150 × 2.1 mm, 2.6 µm, Phenomenex, Aschaffenburg, Germany). Mobile phases were H<sub>2</sub>O with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient elution was: 10% B (0–2 min), 10–80% B (2–8 min), 80–100% B (8–20 min), followed by re-equilibration. Flow rate was 0.3 mL/min, injection volume 5 µL. Electrospray ionization was used in positive and negative mode at ±4.5 kV depending on fragmentation with a collision energy of ±30 V. N<sub>2</sub> was used as nebulizing gas (3 L/min) and drying gas (10 L/min). The desolvation line and heat block temperatures were set to 150°C and 350°C, respectively. Ar was used as collision gas at 230 kPa. Multiple Reaction Monitoring (MRM) was applied in LabSolutions (v5.99 SP2, Shimadzu). A list of analyzed polyphenolic substances and m/z values is shown in Table S2. Polyphenols were put in the categories: flavan-3-ols, flavones, flavonoids, lignans, phenolic acids, phenolic aldehydes and stilbenes. At least one representative standard per polyphenolic class was used for quantification, which are shown in Table S3. Signal-to-noise ratio thresholds were used for limits of detection (LOD) ( $S/N \geq 3$ ) and quantification (LOQ) ( $S/N \geq 10$ ) determination. Data evaluation and processing was done in Skyline (v23.0) (Pino et al., 2020).

## **2.5. Secondary Lipid Oxidation Product Analysis by SPME-GC-MS**

Volatiles were determined via solid-phase microextraction (SPME) coupled with GC-MS based on Guillén et al. (2005) and Holler et al. (2023) (Holler et al., 2023). 2.5 g oil was transferred into 20 mL amber headspace vials, equilibrated for 15 min, and extracted using a

DVB/CAR/PDMS fiber. Volatiles were analyzed using a Shimadzu GCMS-QP2010 Ultra equipped with a ZB-WAX column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m). Oven conditions were 40°C (5 min), ramped at 4°C/min to 280°C. The injector was set to 220°C in splitless mode (10 min fiber desorption). Mass spectra were recorded over  $m/z$  40–400 at 70 eV. Compounds were identified using the NIST library (score >0.85), and key volatiles as hexanal, 1-hexanol, 2-heptanone and 2-pentanone were quantified by calibration curves, which are shown in Table S4. GC–MS data were further processed using OpenChrom software (v1.5.0).

## **2.6. Biomarker Identification and Statistical Analysis**

To identify robust biomarkers indicative of oil deterioration during storage, systematic multivariate statistical and ML approaches were applied to the data obtained from fatty acids (GC-FID), polyphenolic substances (LC-MS/MS), and secondary lipid oxidation products (SPME-GC-MS). Biomarkers were defined as substances where concentrations changed significantly and consistently with storage time. All scripts and analysis pipelines used for biomarker identification are available via GitHub (<https://github.com/TobiPointi/Deterioration-Biomarker-Analysis-in-Cold-Pressed-Oils.git>).

### **2.6.1. Data Normalization and Preprocessing**

For fatty acid data, centered log-ratio (CLR) transformation was applied to account for compositional constraints (Gloor et al., 2017). All datasets were normalized using z-score scaling (mean-centered and divided by standard deviation). Missing values were imputed using forward- and backward-filling strategies. Principal Component Analysis (PCA) was used to visualize sample clustering and variance explanation.

### **2.6.2. Multivariate Analysis and Feature Selection**

Three core statistical models were used for feature selection. Least Absolute Shrinkage and Selection Operator (LASSO) Regression was used to penalize less informative variables

(Ranstam & Cook, 2018). The regularization parameter  $\alpha$  was dynamically adapted (typically 0.01), and a significance threshold of 20% of the maximum absolute coefficient was applied. Random Forest Regression (RFR) is an ensemble ML method that was applied to rank features by their importance in predicting storage time (Breiman, 2001; Ho, 1995). A total of 100 estimators were used, and a dynamic significance threshold of 10% of the maximum importance score was applied. RFR is used as a key tool in feature ranking and biomarker discovery in foodomics and other high-dimensional datasets (Li et al., 2024). Spearman correlation as a non-parametric test between compound concentrations and storage time was calculated to assess monotonic trends. Each compound was evaluated across all three models per oil type and compound class (fatty acids, polyphenols, volatiles).

### **2.6.3. Composite Scoring and Ranking**

A balanced weighting was applied to reflect both model robustness and complementarity. RFR and LASSO were prioritized (40% each) as established feature selection approaches in high-dimensional biomarker discovery, together with correlation (20%) due to its univariate nature and greater susceptibility to spurious associations. Each metric was normalized to a 0–1 scale using min–max normalization, and the final biomarker score was calculated for every substance across all oil types. The highest-scoring compounds were considered the most reliable indicators of oxidative deterioration.

## **2.7. Statistical Testing**

Statistical tests were applied in GraphPad Prism 10.4.1. Outliers were screened using Grubbs' test. One-way ANOVA followed by Tukey's post-hoc test was used to assess significance of concentration changes across time points. For non-parametric comparisons (e.g., day 0 vs day 168), the Mann–Whitney U test was applied. Results were considered statistically significant at  $p$ -values  $< 0.05$ .

### 3. Results

#### 3.1 Fatty Acid Composition by GC-FID

To provide a baseline for subsequent degradation biomarker analysis, the fatty acid composition of the six analyzed cold-pressed oils was determined over 168 days of storage. This characterization is essential, as the fatty acid proportions of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) directly influence oxidative susceptibility (Gharby et al., 2025).

##### 3.1.1 Initial Fatty Acid Distribution

The initial fatty acid composition (day 0) of the six cold-pressed oils black cumin (BC), high-oleic sunflower (HOSF), canola (CA), sunflower (SF), linseed (LS), and hempseed (HS) is shown in Fig. S2. The profiles varied substantially among the oils.

BC was characterized by a high linoleic acid content ( $67.87 \pm 4.27\%$ ), together with palmitic acid ( $14.31 \pm 0.99\%$ ). CA contained high oleic acid ( $55.40 \pm 2.31\%$ ), linoleic acid ( $25.84 \pm 1.03\%$ ), and  $\alpha$ -linolenic acid ( $11.28 \pm 0.51\%$ ). SF was dominated by linoleic acid ( $63.56 \pm 2.35\%$ ). HOSF showed very high oleic acid levels ( $83.10 \pm 3.87\%$ ), as expected for this type of seed. LS exhibited the highest  $\alpha$ -linolenic acid content ( $65.37 \pm 4.02\%$ ), while HS presented a balanced polyunsaturated profile with linoleic acid ( $60.02 \pm 2.60\%$ ) and  $\alpha$ -linolenic acid ( $22.00 \pm 1.16\%$ ).

##### 3.1.2 Changes in Fatty Acids During Storage

Significant changes in fatty acid composition occurred over the 168-day storage period under simulated household conditions, primarily reflecting oxidative degradation of unsaturated fatty acids. The magnitude and kinetics of these changes differed by oil type. The fatty acid degradation data is shown in Figure 1.

In BC, linoleic acid decreased by 66.5% over the storage period, while saturated fatty acids



(SFAs) remained more stable. CA showed decreases of 61.5% in linoleic acid, 55.9% in  $\alpha$ -linolenic acid, and 28.2% in oleic acid. SF exhibited a 57.7% reduction in linoleic acid, while HOSF displayed a 40.8% decline in oleic acid, with smaller changes in PUFAs. LS, initially rich in  $\alpha$ -linolenic acid, showed a 55.8% decline and a 53.6% reduction in linoleic acid, while MUFAs and SFAs remained relatively stable. HS showed decreases in both linoleic and  $\alpha$ -linolenic acid, whereas oleic acid and SFAs remained constant. Overall, oils with higher PUFA content (BC, LS, HS) exhibited the steepest reductions in fatty acids, while oils with lower PUFA proportions (CA, SF, HOSF) showed more moderate declines.

### **3.2 Polyphenol Analysis by LC-MS/MS**

Polyphenols in edible oils serve as important antioxidants that inhibit and delay lipid oxidation by scavenging free radicals and chelating pro-oxidative metals, thus playing a key role in shelf-life stability. Their profiles and concentrations were analyzed via LC-MS/MS in MRM mode.

#### **3.2.1 Initial Total Polyphenol Content and Class Distribution**

At the beginning of storage, the six cold-pressed oils showed clear differences in total polyphenol content as shown in Fig. S3. BC contained the highest levels with  $745.1 \pm 227.2$   $\mu\text{g/g}$ , significantly exceeding all other oils ( $p < 0.05$ ). The other oils (CA, SF, HOSF, LS, HS) showed lower concentrations ranging from 68.9 to 150.7  $\mu\text{g/g}$ .

Polyphenols were grouped into phenolic acids, flavonoids, flavones, flavan-3-ols, lignans, and stilbenes. The relative distribution of these classes is shown in Fig. S4. BC exhibited the most complex composition, dominated by phenolic acids (80%), with smaller contributions from flavonoids (12.5%), flavones (5.5%), and small amounts of flavan-3-ols and lignans. Stilbenes and phenolic aldehydes were only detected in BC. CA also contained predominantly phenolic acids (53%) and a notable fraction of flavones (28.1%). SF showed the highest proportion of flavones (51.6%). LS contained high flavone levels (44.3%) along with some lignans (7%). HS

displayed a more diverse profile, with phenolic acids (43.8%), flavonoids (24.4%), flavones (18.6%), and lignans (9%).

### 3.2.2. Polyphenol Degradation over Storage Time

The antioxidant activity of polyphenols leads to their gradual decline during storage, as these compounds neutralize lipid-derived radicals and chelate metal ions (Shahidi & Ambigaipalan, 2015). All six cold-pressed oils showed a significant decrease in total polyphenol content across the 168-day storage period, although the extent and rate of decline varied by oil type, which is shown in Figure 2. BC exhibited the steepest decline, with a marked reduction already evident at day 3. By day 168, 95% of the initial polyphenols were degraded, resulting in  $34.8 \pm 17.5$   $\mu\text{g/g}$  oil at the end of storage. Despite this loss, BC maintained the highest residual polyphenol concentration throughout the study. CA showed a gradual, near-linear decrease over time. HOSF, with lower initial phenolic levels, exhibited an early reduction within the first 3 days. SF, LS, and HS followed similar patterns, with significant decreases detected from day 3 onward. By day 84, all three had lost more than 50% of their initial polyphenol content.

In most oils, phenolic acids exhibited the earliest and steepest decreases, significant from day 3 onward, whereas flavonols, flavones, and lignans showed comparatively slower declines. Figure 3 provides a heatmap of all 48 quantified polyphenols in BC, grouped by class, across storage. Phenolic acids were initially dominant and exhibited the steepest declines during storage. For instance, p-coumaric acid decreased from 538.2 to 13.2  $\mu\text{g/g}$  (−97.5%), and quinic acid from 25.3 to 2.42  $\mu\text{g/g}$  (−90%). The visualization highlights the rapid depletion of phenolic acids within the first weeks, while flavonoids, lignans, and stilbenes persisted longer.

The full dataset of all quantified polyphenolic compounds is provided in Table S5. In CA, sinapic acid exhibited an early transient increase before declining to low residual levels by day 168 (−80%). Ferulic acid and ellagic acid also decreased strongly. Herbacetin, as the most

abundant flavone, decreased from 19.1 to 1.41  $\mu\text{g/g}$  (–93%). In SF, ellagic acid dropped from 16.7 to 0.45  $\mu\text{g/g}$  (–97%), while quercetin-3-O-glucoside decreased from 1.39 to 0.13  $\mu\text{g/g}$  (–91%) and in HOSF, ellagic acid decreased from 10.4 to 0.47  $\mu\text{g/g}$  (–95%). Caffeic acid and its hexoside showed rapid early-phase depletion. In LS, hydroxycinnamates showed rapid loss, with p-coumaric acid decreasing from 6.07  $\mu\text{g/g}$  to 0.53  $\mu\text{g/g}$  by day 168 (–92%). Several linseed specific lignans such as secoisolariciresinol also diminished by about 80% over the six months study period. In HS, p-coumaric acid decreased from 7.2 to 0.39  $\mu\text{g/g}$  (–95%), while kaempferol conjugates and lignans showed more gradual mid-term declines.

Overall, large quantitative differences and specific polyphenolic fingerprints were observed across oils, with BC showing the highest initial levels but also the steepest absolute reductions, while CA, SF, HOSF, LS and HS exhibited distinct compound-specific degradation patterns. Together, these findings demonstrate that polyphenolic degradation is not only concentration-dependent but also matrix-specific. Phenolic acids generally showed the fastest decay, while flavonoids and lignans tended to degrade more slowly. These insights support the strategic use of specific polyphenols as early or mid-term markers of oil stability.

### **3.3 Secondary Lipid Oxidation Products via SPME-GC-MS**

Volatile compounds formed by secondary lipid oxidation are key indicators of oil deterioration and were monitored via SPME-GC-MS. Among the analyzed oils hexanal was quantified over 168 days as shown in Figure 4. The formation was oil-dependent. LS and HS showed the strongest increases. LS rose to 18.3 ppm after 42 days, while HS increased to 6.7 ppm after 28 days already, reached 28.9 ppm after 42 days, and remained >24 ppm thereafter. Both oils followed between day 14 and day 42 an exponential, pseudo-first-order pattern in the propagation phase:

$$\textbf{Equ. 1: } C_{LS}(t) = 0.08 * e^{0.15*(t-14)}, (R^2 = 0.998)$$

$$\textbf{Equ. 2: } C_{HS}(t) = 0.11 * e^{0.14*(t-14)}, (R^2 = 0.991)$$

where C(t) is the hexanal concentration [ppm] at time t [days].

By contrast, the other oils stayed an order of magnitude lower over the whole study. SF exhibited a transient maximum of 3.9 ppm after 84 days followed by a decrease to 1.28 ppm at day 168. BC rose modestly to 1.45 ppm by day 112, HOSF gradually to 0.96 ppm and CA to 2.13 ppm by the end of storage.

Across the different oil types tested distinct fingerprints for unsaturated alkenals (e.g., (E)-2-nonenal, (E)-2-decenal), conjugated dienals (2,4-heptadienal, 2,4-decadienal), saturated aldehydes (heptanal, nonanal), furans (2-pentyl-furan, 5-ethyl-2(5)-furanone), allylic alcohols (2-octen-1-ol, 2-decen-1-ol, 1-octen-3-ol), and advanced markers as e.g. 4,5-epoxy-(E)-2-decenal were evaluated. All detected and identified secondary lipid oxidation compounds over time of storage expressed either as quantified concentrations [ppm] or semi-quantified area under the curve [AuC] values are reported in Table S6 and S7. In total 20 secondary lipid oxidation products could be identified.

In BC, nonanal and the electrophilic aldehyde 4,5-epoxy-(E)-2-decenal increased most prominently, showing 10.3- and 13.9-fold rises, respectively, by the end of storage, while heptanal rose 6.5-fold. Unsaturated aldehydes such as (E)-2-decenal and (E)-2-octenal accumulated steadily, whereas 5-ethyl-2(5)-furanone showed a transient maximum around day 112 (3.1-fold increase). In CA, conjugated dienals were dominant, with 2,4-decadienal rising 13-fold by day 112. Moderate increases were also observed for (E)-2-nonenal, 2,4-heptadienal and heptanal. SF showed a similar aldehyde profile, with a 13.1-fold increase in 2,4-decadienal and 3.7-fold in (E)-2-nonenal peaking after 112 days, while other dienals rose earlier. In HOSF,

nonanal was the most dynamic marker, increasing nearly 5-fold within 42 days. Decanal also rose sharply by day 84, while (E)-2-decenal increased mid-storage. LS was characterized by a rise of 2-pentyl-furan, which peaked at day 112 with 28.9-fold higher levels and remained elevated thereafter. Conjugated dienals such as 2,4-heptadienal and 2,4-decadienal also accumulated, together with nonanoic acid, while 1-octen-3-ol declined rapidly. In HS, (E)-2-heptenal showed the most increase by 42.7-fold by day 84. Substantial increases were also seen for 2-pentyl-furan, 2,4-decadienal, and nonanoic acid, alongside moderate rises in (E)-2-nonenal, heptanal and 5-ethyl-2(5)-furanone. These measurements highlight distinct, oil-specific volatile fingerprints that underpin the biomarker selection discussed in the next section.

### **3.4 Biomarker Identification from Oil-Specific Fingerprints**

To identify reliable indicators of oil deterioration, a comprehensive biomarker analysis was conducted using combined datasets from fatty acids (GC-FID), polyphenols (LC-MS/MS), and secondary lipid oxidation products (GC-MS). Each dataset was subjected to LASSO, RFR, and Spearman correlation. These were normalized (z-score), weighted and aggregated to generate a final ranking of potential biomarkers per oil type. This integrative strategy revealed distinct fingerprints of deterioration for each oil, comprising substances with the most time-responsive behavior across chemical classes. PCAs were performed on each dataset of fatty acids, polyphenols and volatiles to assess clustering patterns and variance explained. Fig. S5 illustrates the PCA scores and loadings for fatty acids in BC, showing a clear separation of storage stages along PC1 and PC2, supporting the robustness of the biomarker selection approach. The top biomarkers per oil type are listed in Table 1.

For BC, linoleic acid was the fatty acid-derived marker with the highest score, reflecting the fatty acid degradation, while 2-decen-1-ol, (E)-2-nonenal and 2-octen-1-ol stood out among volatile oxidation products. Additionally, matairesinol emerged as the most relevant polyphenol

marker, confirming the susceptibility of lignans to degradation in this oil type. For CA, the lipid oxidation products 2,4-decadienal and 2,4-heptadienal were most prominent, followed by the fatty acids oleic acid, linoleic acid, and  $\alpha$ -linolenic acid. Sinapic acid was highlighted as the key polyphenol biomarker, aligning with its well-known role in canola oil stability (Koski et al., 2003). In SF, the volatile aldehydes 2,4-decadienal and 2,4-heptadienal dominated the biomarker fingerprint, while linoleic acid and oleic acid were consistent fatty acid markers. Quercetin-3-O-glucuronic acid was identified as the most reliable polyphenolic marker. In HOSF, decanal dominated the biomarker profile, indicating its strong link to oxidative rancidity in this oil, while caffeic acid and quinic acid were identified as the top polyphenol-related markers. For LS,  $\alpha$ -linolenic acid was the strongest biomarker, consistent with the high susceptibility of this highly unsaturated fatty acid to oxidation. Volatile alcohols such as 1-hexanol and 1-octen-3-ol were also highlighted, alongside p-coumaric acid as polyphenolic marker. Finally, HS was best described by the decline of linoleic acid, as the strongest biomarker across all oils (weighted score = 1.0). In addition, 1-hexanol, 2,4-decadienal and (E)-2-heptenal contributed to the volatile fingerprint, while chlorogenic acid was identified as the most significant polyphenol marker.

Beyond the top biomarkers found, the full weighted score tables for fatty acids, polyphenols, and secondary lipid oxidation products are in Tables S1–S3. Overall, fatty acids tended to receive higher weighted scores, reflecting their strong and consistent contribution to oil deterioration across storage conditions. In contrast, polyphenols generally showed lower scores, which can be explained by their high chemical diversity and oil-specific relevance rather than universal predictive power. Secondary lipid oxidation products occupied an intermediate position, with several aldehydes and alcohols emerging as robust markers in specific oils. These patterns underline that a combined fingerprint across fatty acids and volatiles provides the most



reliable biomarker set, while polyphenols add important oil-specific resolution.

## **4. Discussion**

This six-month storage study demonstrated that the oxidative deterioration of six cold-pressed vegetable oils is strongly influenced by their fatty acid composition, polyphenol content and the formation of secondary lipid oxidation products. By integrating GC-FID, LC-MS/MS, and GC-MS data with ML-based ranking approaches (LASSO, RFR and Spearman), it was possible to identify oil-type-specific biomarker fingerprints that provide mechanistic insights into oxidative stability and deterioration. Unlike most previous storage studies, which relied on dark glass or accelerated conditions, our design simulated household conditions by using transparent PET bottles exposed to light at ambient temperature.

### **4.1 Role of Fatty Acid Composition in Oxidative Stability**

Fatty acids consistently received the highest biomarker scores across nearly all oils, highlighting their central role as primary substrates for lipid oxidation. This outcome aligns with the well-established association between PUFA content and reduced oxidative stability (Choe & Min, 2006; Koch et al., 2023). Oils rich in  $\alpha$ -linolenic acid (LS, HS) or linoleic acid (BC, SF) deteriorated fastest, reflected by steep PUFA losses and sharp rises in aldehydic volatiles (e.g., hexanal, 2-pentylfuran). Similar kinetics have been reported for flaxseed and other n-3-rich oils stored under light/air exposure (Cui et al., 2024; Grajzer et al., 2020; Tanouti et al., 2012). In contrast, high-oleic sunflower oil (HOSF) demonstrated markedly higher oxidative stability, consistent with its reduced PUFA fraction and enrichment in oleic acid. Previous studies have also shown that high-oleic cultivars maintain lower peroxide values and volatile aldehyde formation compared to conventional sunflower oil (Holler et al., 2023; Pignitter et al., 2014). Collectively, these results confirm that baseline susceptibility is largely encoded by the degree of unsaturation.

## 4.2 Polyphenol Degradation

Previous olive oil studies reported 40–60% polyphenol losses over 12 months in darkness (Castillo-Luna et al., 2021). By contrast under light conditions, BC with the highest initial phenolic concentration (745 µg/g oil) exhibited rapid early loss of -85% of its major phenolic substance p-coumaric acid after just 14 days, underscoring the accelerating effect of light exposure and transparent packaging. This pattern mirrors prior observations that classically reactive phenolics act as sacrificial antioxidants during propagation/termination steps (Böhm et al., 2013; Kanner, 2007; Toma et al., 2015) and agrees with earlier findings in black cumin seed extracts, where phenolic acids were consumed rapidly during oxidative challenges (Ahmad et al., 2013).

Despite its steep decline, BC retained the highest residual phenolic levels after 6 months, supporting its relative stability compared to other PUFA-rich oils. Similar roles of specific phenolics were observed in other oils. Caffeic acid in HOSF and sinapic acid in CA were identified as relevant biomarkers, both of which have been reported as effective antioxidants in lipid matrices (Shahidi & Ambigaipalan, 2015). In canola oil, sinapic acid and its derivatives are well-documented contributors to oxidative stability, though they also undergo rapid photooxidative degradation in transparent packaging (Koski et al., 2003).

Taken together, our findings show that fatty acid composition sets the baseline susceptibility to oxidation, while polyphenols modulate the rate and trajectory of deterioration. Oils with both high PUFA content (e.g., HS, LS) and lower phenolic reserves were most vulnerable, whereas oils with either high MUFA (HOSF) or high phenolic content (BC) resisted deterioration more effectively.

### 4.3 Secondary Lipid Oxidation Products as Functional Biomarkers

Secondary lipid oxidation products, particularly volatile aldehydes, emerged as strong and oil-type-specific markers. Compounds such as nonanal, 2,4-decadienal, and (E)-2-nonenal showed marked increases in several oils, consistent with their low odor thresholds and established role as indicators of rancidity (Crackel et al., 1988). Hexanal, often used as a universal marker of PUFA oxidation (Jacobsen et al., 2008; Sharma et al., 2022), accumulated exponentially in LS and HS. The pseudo-first-order fit was valid during the propagation phase from day 14 to 42, reflecting rapid exponential growth in line with their high PUFA content, while later stages seemed to deviate due to substrate depletion and secondary reactions. These kinetics support previous reports that hexanal associates with sensory rejection in PUFA-rich oils and provide quantitative evidence that ~1 ppm may serve as a practical threshold value for early detection of rancidity in household-stored oils (Clarke et al., 2020; Gharby et al., 2025; Warner & Eskin, 1995). Nevertheless, as shown in olive and walnut oil studies (Ampofo et al., 2022), hexanal alone cannot fully explain rancidity perception across matrices.

### 4.4 Systematic Biomarker Identification

By combining datasets from fatty acids, polyphenols, and volatiles, and applying ML-based feature selection, this study provides a new framework for biomarker discovery in edible oils. Similar multivariate and data-driven approaches have been successfully applied in metabolomics and foodomics for biomarker identification (Li et al., 2024; W. Zhang et al., 2025). The resulting hierarchy in our study showing that fatty acids dominating biomarker scores, followed by secondary volatiles, and polyphenols contributing lower but meaningful scores reflects the mechanistic sequence of deterioration. PUFA oxidation initiates instability, antioxidant depletion modulates the rate, and aldehyde accumulation serves as a terminal

indicator of rancidity. The oil-type-specific fingerprints emphasize distinct vulnerabilities, such as rapid polyphenol depletion in black cumin oil, steep PUFA oxidation in linseed and hempseed oils, and aldehyde accumulation in sunflower and canola oils.

#### **4.5 Study Limitations and Outlook**

The novelty of this study lies in the systematic comparison of six cold-pressed oils from three different suppliers under household conditions to identify oil-type specific deterioration biomarkers. Our study design allowed us to capture the rapid degradation of light-sensitive antioxidants, with polyphenol losses already evident in the early days of storage. This effect was repeatedly demonstrated in other edible oils stored under illumination (Holler et al., 2023; Pignitter et al., 2014). By integrating chemical classes across methods and applying statistical learning, our study extends this work and demonstrates that deterioration cannot be reliably predicted by a single marker. Instead, robust oil-type-specific fingerprints emerge, offering a more nuanced and reliable approach to shelf-life assessment.

Limitations include the absence of other packaging materials (e.g., amber glass), which are known to provide greater light protection (Castillo-Luna et al., 2021; Grajzer et al., 2020). In addition, the study did not include direct sensory validation or accelerated storage tests, which would strengthen correlations between chemical markers and consumer perception. Future work should therefore validate the proposed biomarker fingerprints across different packaging materials and link them to sensory rejection thresholds in order to establish consumer-relevant cutoffs.

## 5. Conclusion

The present study provides an integrated comprehensive analysis of lipid oxidation in six cold-pressed oils, combining big data sets of GC-FID, LC-MS/MS and GC-MS measurements to identify oil-type-specific deterioration biomarkers. Fatty acid composition primarily determines oxidative susceptibility, while phenolic antioxidants modulate the rate and secondary volatiles mark the endpoint of degradation. The resulting fingerprints over half a year of storage capture the mechanistic sequence of deterioration and explain oil-specific oxidative stability differences under realistic household storage conditions.

In this study determined oil-type-specific deterioration biomarkers are not only beneficial for further knowledge of lipid oxidation pathways but also lay the groundwork for predictive shelf-life models. Integration of the proposed biomarkers with rapid, non-invasive screening technologies such as near-infrared spectroscopy (NIR) or electronic noses (e-nose), combined with ML modeling could offer promising applications for real-time quality, monitoring in industrial and domestic contexts. Such methods could significantly contribute to reducing food waste by enabling early detection of oxidative spoilage.

### CRedit authorship contribution statement

Tobias Pointner: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. Philipp Steinlechner: Methodology, Formal analysis, Investigation, Data curation, Visualization. Marc Pignitter: Conceptualization, Resources, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

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## Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT (GPT-5, OpenAI, San Francisco, USA) to improve the structure and language of the manuscript. It was also used to assist with debugging and code error handling. DeepL.com (v. 3.5, DeepL SE, Cologne, Germany) was used for language refinement and translation of sections originally drafted in German. After using these tools, the authors carefully reviewed and edited all content to ensure accuracy and take full responsibility for the integrity and originality of the published work.



## References

- Ahmad, Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., Damanhour, Z. A. and Anwar, F. (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*, 3(5), 337–352. [https://doi.org/10.1016/S2221-1691\(13\)60075-1](https://doi.org/10.1016/S2221-1691(13)60075-1)
- Ampofo, J., Grilo, F. S., Langstaff, S. and Wang, S. C. (2022). Oxidative Stability of Walnut Kernel and Oil: Chemical Compositions and Sensory Aroma Compounds. *Foods*, 11(19). <https://doi.org/10.3390/foods11193151>
- Bayram, I., Parra-Escudero, C., Decker, E. A. and Lu, J. (2024). Mathematical Modeling of Alpha-Tocopherol Early Degradation Kinetics to Predict the Shelf-Life of Bulk Oils. *Journal of Agricultural and Food Chemistry*, 72(9), 4939–4946. <https://doi.org/10.1021/acs.jafc.3c08272>
- Böhm, T., Berger, H., Nejabat, M., Riegler, T., Kellner, F., Kuttke, M., Sagmeister, S., Bazanella, M., Stolze, K., Daryabeigi, A., Binther, N., Murkovic, M., Wagner, K.-H., Schulte-Hermann, R., Rohr-Udilova, N., Huber, W. and Grasl-Kraupp, B. (2013). Food-derived peroxidized fatty acids may trigger hepatic inflammation: A novel hypothesis to explain steatohepatitis. *Journal of Hepatology*, 59(3), 563–570. <https://doi.org/10.1016/j.jhep.2013.04.025>
- Breiman, L. (2001). Random Forests. *Machine Learning*, 45(1), 5–32. <https://doi.org/10.1023/A:1010933404324>
- Castillo-Luna, A., Criado-Navarro, I., Ledesma-Escobar, C. A., López-Bascón, M. A. and Priego-Capote, F. (2021). The decrease in the health benefits of extra virgin olive oil during storage is conditioned by the initial phenolic profile. *Food Chemistry*, 336, 127730. <https://doi.org/10.1016/j.foodchem.2020.127730>
- Choe, E. and Min, D. B. (2006). Mechanisms and Factors for Edible Oil Oxidation. *Comprehensive Reviews in Food Science and Food Safety*, 5(4), 169–186. <https://doi.org/10.1111/j.1541-4337.2006.00009.x>
- Clarke, H. J., O'Sullivan, M. G., Kerry, J. P. and Kilcawley, K. N. (2020). Correlating Volatile Lipid Oxidation Compounds with Consumer Sensory Data in Dairy Based Powders during Storage. *Antioxidants (Basel, Switzerland)*, 9(4), 338. <https://doi.org/10.3390/antiox9040338>
- Crackel, R. L., Gray, J. I., Booren, A. M., Pearson, A. M. and Buckley, D. J. (1988). Effect of Antioxidants on Lipid Stability in Restructured Beef Steaks. *Journal of Food Science*, 53(2), 656–657. <https://doi.org/10.1111/j.1365-2621.1988.tb07780.x>
- Cui, F., Liu, M., Li, X., Du Wang, Ma, F., Yu, L., Hu, C., Li, P. and Zhang, L. (2024). Gas chromatography ion mobility spectroscopy: A rapid and effective tool for monitoring oil oxidation. *Food Research International*, 176, 113842. <https://doi.org/10.1016/j.foodres.2023.113842>
- Geng, L., Liu, K. and Zhang, H. (2023). Lipid oxidation in foods and its implications on proteins. *Frontiers in Nutrition*, 10, 1192199. <https://doi.org/10.3389/fnut.2023.1192199>
- Gharby, S., Asbbane, A., Nid Ahmed, M., Gagour, J., Hallouch, O., Oubannin, S., Bijla, L., Goh, K. W., Bouyahya, A. and Ibourki, M. (2025). Vegetable oil oxidation: Mechanisms, impacts on quality, and approaches to enhance shelf life. *Food Chemistry: X*, 28, 102541. <https://doi.org/10.1016/j.fochx.2025.102541>
- Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V. and Egozcue, J. J. (2017). Microbiome Datasets Are Compositional: And This Is Not Optional. *Frontiers in Microbiology*, 8, 2224. <https://doi.org/10.3389/fmicb.2017.02224>
- Grajzer, M., Szmalczyk, K., Kuźmiński, Ł., Witkowski, M., Kulma, A. and Prescha, A. (2020). Characteristics and Antioxidant Potential of Cold-Pressed Oils-Possible Strategies to Improve Oil Stability. *Foods*, 9(11). <https://doi.org/10.3390/foods9111630>
- Grüneis, V., Fruehwirth, S., Zehl, M., Ortner, J., Schamann, A., König, J. and 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(36), 10174–10184. <https://doi.org/10.1021/acs.jafc.9b03601>
- Guillén, M. D., Cabo, N., Ibargoitia, M. L. and Ruiz, A. (2005). Study of both sunflower oil and its headspace throughout the oxidation process. Occurrence in the headspace of toxic oxygenated aldehydes. *Journal of Agricultural and Food Chemistry*, 53(4), 1093–1101. <https://doi.org/10.1021/jf0489062>
- Haque, M., Khan, F. N., Yadav, S., Kaur, V., Kaushik, N., Kumar, A. and Langyan, S. (2024). Chemistry, bioactivity, and health effects of linseed food products as nutraceutical and dietary supplements. In *Linseed* (pp. 79–90). Elsevier. <https://doi.org/10.1016/B978-0-443-15439-3.00012-6>
- Ho, T. K. (1995). Random decision forests. In *Proceedings of 3rd International Conference on Document Analysis and Recognition* (pp. 278–282). IEEE Comput. Soc. Press. <https://doi.org/10.1109/ICDAR.1995.598994>
- Holler, M., Alberdi-Cedeño, J., Auñón-Lopez, A., Pointner, T., Martínez-Yusta, A., König, J. and Pignitter, M. (2023). Polylactic acid as a promising sustainable plastic packaging for edible oils. *Food Packaging and Shelf Life*, 36, 101051. <https://doi.org/10.1016/j.fpsl.2023.101051>
- Jacobsen, C., Let, M. B., Nielsen, N. S. and Meyer, A. S. (2008). Antioxidant strategies for preventing oxidative flavour deterioration of foods enriched with n-3 polyunsaturated lipids: a comparative evaluation. *Trends in Food Science & Technology*, 19(2), 76–93. <https://doi.org/10.1016/j.tifs.2007.08.001>
- Kanner, J. (2007). Dietary advanced lipid oxidation endproducts are risk factors to human health. *Molecular Nutrition & Food Research*, 51(9), 1094–1101. <https://doi.org/10.1002/mnfr.200600303>
- Karami, H., Rasekh, M. and Mirzaee – Ghaleh, E. (2020). Comparison of chemometrics and AOCS official methods for predicting the shelf life of edible oil. *Chemometrics and Intelligent Laboratory Systems*, 206, 104165. <https://doi.org/10.1016/j.chemolab.2020.104165>
- Koch, E., Löwen, A., Kampschulte, N., Plitzko, K., Wiebel, M., Rund, K. M., Willenberg, I. and Schebb, N. H. (2023). Beyond Autoxidation and Lipoxygenases: Fatty Acid Oxidation Products in Plant Oils. *Journal of Agricultural and Food Chemistry*, 71(35), 13092–13106. <https://doi.org/10.1021/acs.jafc.3c02724>
- Koski, A., Pekkarinen, S., Hopia, A., Whl, K. and Heinonen, M. (2003). Processing of rapeseed oil: effects on sinapic acid derivative content and oxidative stability. *European Food Research and Technology*, 217(2), 110–114. <https://doi.org/10.1007/s00217-003-0721-4>
- Lall, R. K., Proctor, A. and 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(4), 309–314. <https://doi.org/10.1007/s11746-009-1359-6>
- Li, D., Bai, L., Wang, R. and Ying, S. (2024). Research Progress of Machine Learning in Extending and Regulating the Shelf Life of Fruits and Vegetables. *Foods*, 13(19). <https://doi.org/10.3390/foods13193025>
- Mehany, T., González-Sáiz, J. M. and Pizarro, C. (2025). Hydroxytyrosol-Infused Extra Virgin Olive Oil: A Key to Minimizing Oxidation, Boosting Antioxidant Potential, and Enhancing Physicochemical Stability During Frying. *Antioxidants*, 14(3). <https://doi.org/10.3390/antiox14030368>
- Parisi, S. (2016). Min Hu and Charlotte Jacobsen (Eds.): Oxidative stability and shelf life of foods containing oils and fats. *Analytical and Bioanalytical Chemistry*, 408(27), 7549–7550. <https://doi.org/10.1007/s00216-016-9875-3>
- Pignitter, M., Stolze, K., Gartner, S., Dumhart, B., Stoll, C., Steiger, G., Kraemer, K. and 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(10), 2297–2305. <https://doi.org/10.1021/jf405736j>

Pino, L. K., Searle, B. C., Bollinger, J. G., Nunn, B., MacLean, B. and MacCoss, M. J. (2020). The Skyline ecosystem: Informatics for quantitative mass spectrometry proteomics. *Mass Spectrometry Reviews*, 39(3), 229–244. <https://doi.org/10.1002/mas.21540>

Pointner, T., Rauh, K., Auñon-Lopez, A., Kostadinović Veličkovska, S., Mitrev, S., Arsov, E. and Pignitter, M. (2024). Comprehensive analysis of oxidative stability and nutritional values of germinated linseed and sunflower seed oil. *Food Chemistry*, 454, 139790. <https://doi.org/10.1016/j.foodchem.2024.139790>

Ranstam, J. and Cook, J. A. (2018). LASSO regression. *British Journal of Surgery*, 105(10), 1348. <https://doi.org/10.1002/bjs.10895>

Shahidi, F. and Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. *Journal of Functional Foods*, 18, 820–897. <https://doi.org/10.1016/j.jff.2015.06.018>

Sharma, A., Bhardwaj, A., Khanduja, G., Kumar, S., Bagchi, S., Kaur, R., Sharma, M., Singla, M., Ravinder, T., Bhondekar, A. P. and Prabhavathi Devi, B. L. A. (2022). Determination of Hexanal Using Static Headspace GC-FID Method and Its Correlation with Oxidative Rancidity in Edible Oils. *Food Analytical Methods*, 15(10), 2652–2663. <https://doi.org/10.1007/s12161-022-02320-4>

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3), 144–158. <https://doi.org/10.5344/ajev.1965.16.3.144>

Tanouti, K., Serghini-Caid, H., Sindic, M., Wathelet, J.-P., Bouseta, A. and Elamrani, A. (2012). Volatile Compounds, Profiles of Virgin Olive Oils Produced In the Eastern Morocco: Oxidative Stability and Sensory Defects. *Journal of Food Research*, 1(4), 194. <https://doi.org/10.5539/jfr.v1n4p194>

Tawfik, M. and Huyghebaert, A. (1999). Interaction of packaging materials and vegetable oils: oil stability. *Food Chemistry*, 64(4), 451–459. [https://doi.org/10.1016/S0308-8146\(97\)00202-1](https://doi.org/10.1016/S0308-8146(97)00202-1)

Toma, C.-C., Olah, N.-K., Vlase, L., Mogoșan, C. and Mocan, A. (2015). Comparative Studies on Polyphenolic Composition, Antioxidant and Diuretic Effects of *Nigella sativa* L. (Black Cumin) and *Nigella damascena* L. (Lady-in-a-Mist) Seeds. *Molecules*, 20(6), 9560–9574. <https://doi.org/10.3390/molecules20069560>

Warner, K. and Eskin, M. (1995). *Methods to Access Quality and Stability of Oils and Fat-Containing Foods*. AOCS Publishing. <https://doi.org/10.4324/9781003040958>

Zhang, W., Huang, H., Wang, L., Lehmann, B. D. and Chen, S. X. (2025). An Integrative Multi-Omics Random Forest Framework for Robust Biomarker Discovery. *BioRxiv*. <https://doi.org/10.1101/2025.03.05.641533>

Table 1: Results of the top biomarker identification for each oil type with weighted score.

| Oil Type                | Substance Type | Substance                     | Weighted Score | Trend over Time |
|-------------------------|----------------|-------------------------------|----------------|-----------------|
| <b>Black cumin oil</b>  | Fatty Acid     | Linoleic acid                 | 0.68           | ↓               |
|                         | Fatty Acid     | Stearic acid                  | 0.65           | ↓               |
|                         | Volatile       | 2-Decen-1-ol                  | 0.55           | ↑               |
|                         | Volatile       | (E)-2-Nonenal                 | 0.54           | ↑               |
|                         | Volatile       | 2-Octen-1-ol                  | 0.42           | ↑               |
|                         | Polyphenol     | Matairesiol                   | 0.28           | ↓               |
| <b>Canola oil</b>       | Volatile       | 2,4-Decadienal                | 0.95           | ↑               |
|                         | Fatty Acid     | Oleic acid                    | 0.89           | ↓               |
|                         | Fatty Acid     | Linoleic acid                 | 0.58           | ↓               |
|                         | Fatty Acid     | $\alpha$ -Linolenic acid      | 0.52           | ↓               |
|                         | Volatile       | 2,4-Heptadienal               | 0.50           | ↑               |
|                         | Polyphenol     | Sinapic acid                  | 0.37           | ↓               |
| <b>Sunflower oil</b>    | Volatile       | 2,4-Decadienal                | 0.91           | ↑               |
|                         | Fatty Acid     | Linoleic acid                 | 0.86           | ↓               |
|                         | Fatty Acid     | Oleic acid                    | 0.57           | ↓               |
|                         | Volatile       | 2,4-Heptadienal               | 0.41           | ↑               |
|                         | Polyphenol     | Quercetin-3-O-glucuronic acid | 0.32           | ↓               |
| <b>HO sunflower oil</b> | Volatile       | Decanal                       | 0.96           | ↑               |
|                         | Fatty Acid     | Stearic acid                  | 0.84           | ↓               |
|                         | Fatty Acid     | Linoleic acid                 | 0.78           | ↓               |
|                         | Polyphenol     | Caffeic acid                  | 0.50           | ↓               |
|                         | Polyphenol     | Quinic acid                   | 0.33           | ↓               |
| <b>Linseed oil</b>      | Fatty Acid     | $\alpha$ -Linolenic acid      | 0.78           | ↓               |
|                         | Volatile       | 1-Hexanol                     | 0.56           | ↑               |
|                         | Fatty Acid     | Oleic acid                    | 0.44           | ↓               |
|                         | Volatile       | 1-Octen-3-ol                  | 0.43           | ↑               |
|                         | Polyphenol     | p-Coumaric acid               | 0.40           | ↓               |
| <b>Hempseed oil</b>     | Fatty Acid     | Linoleic acid                 | 1.000          | ↓               |
|                         | Volatile       | 1-Hexanol                     | 0.46           | ↑               |
|                         | Volatile       | 2,4-Decadienal                | 0.42           | ↑               |
|                         | Volatile       | (E)-2-Heptenal                | 0.36           | ↑               |
|                         | Polyphenol     | Chlorogenic acid              | 0.33           | ↓               |

**Figure Captions**

**Fig. 1:** Changes in fatty acid composition [mg/g oil] during 168 days of storage for the six cold-pressed oils under household conditions: **(A)** black cumin, **(B)** canola, **(C)** sunflower, **(D)** high-oleic sunflower, **(E)** linseed and **(F)** hempseed oil. One-way ANOVA with Tukey's *post hoc* test was conducted. Statistical differences are indicated with different lowercase letters ( $p < 0.05$ ). Each data point represents the mean  $\pm$  **SD**,  $n \geq 9$ .

**Fig. 2:** Change of total polyphenol content [ $\mu\text{g/g}$  oil] in six cold-pressed oils during 168 days of storage, measured via LC-MS/MS. **(A)** black cumin, **(B)** canola, **(C)** sunflower, **(D)** high-oleic sunflower, **(E)** linseed and **(F)** hempseed oil. One-way ANOVA with Tukey's *post hoc* test was conducted. Statistical differences are indicated with different lowercase letters ( $p < 0.05$ ). Each data point represents the mean  $\pm$  **SD**,  $n \geq 9$ .

**Fig. 3:** Heatmap of quantified polyphenols in black cumin oil across six months storage. The color scale indicates different concentrations [ $\mu\text{g/g}$  oil] and the different specific polyphenolic substances identified and quantified were put into polyphenolic classes. Crossed-out cells are  $\leq$  LOQ. Values are shown as mean,  $n \geq 9$ .

**Fig. 4:** Hexanal concentration in ppm over 168 days of storage in the six cold-pressed oils analyzed. One-way ANOVA with Tukey's *post hoc* test was conducted. Statistical differences are indicated with different lowercase letters ( $p < 0.05$ ). The data points represent values of mean  $\pm$  **SD**,  $n \geq 9$ .

Fig. 1:

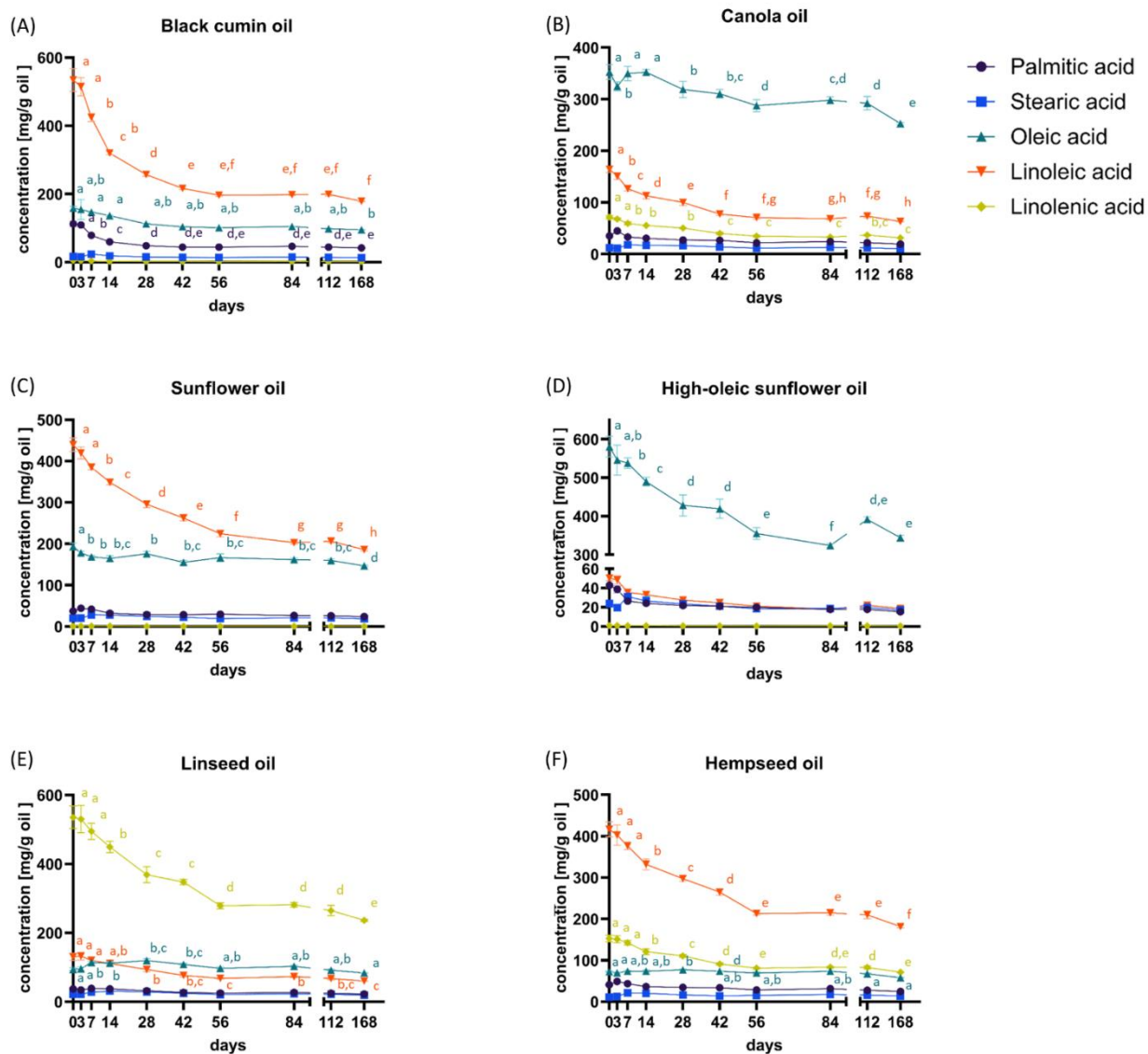
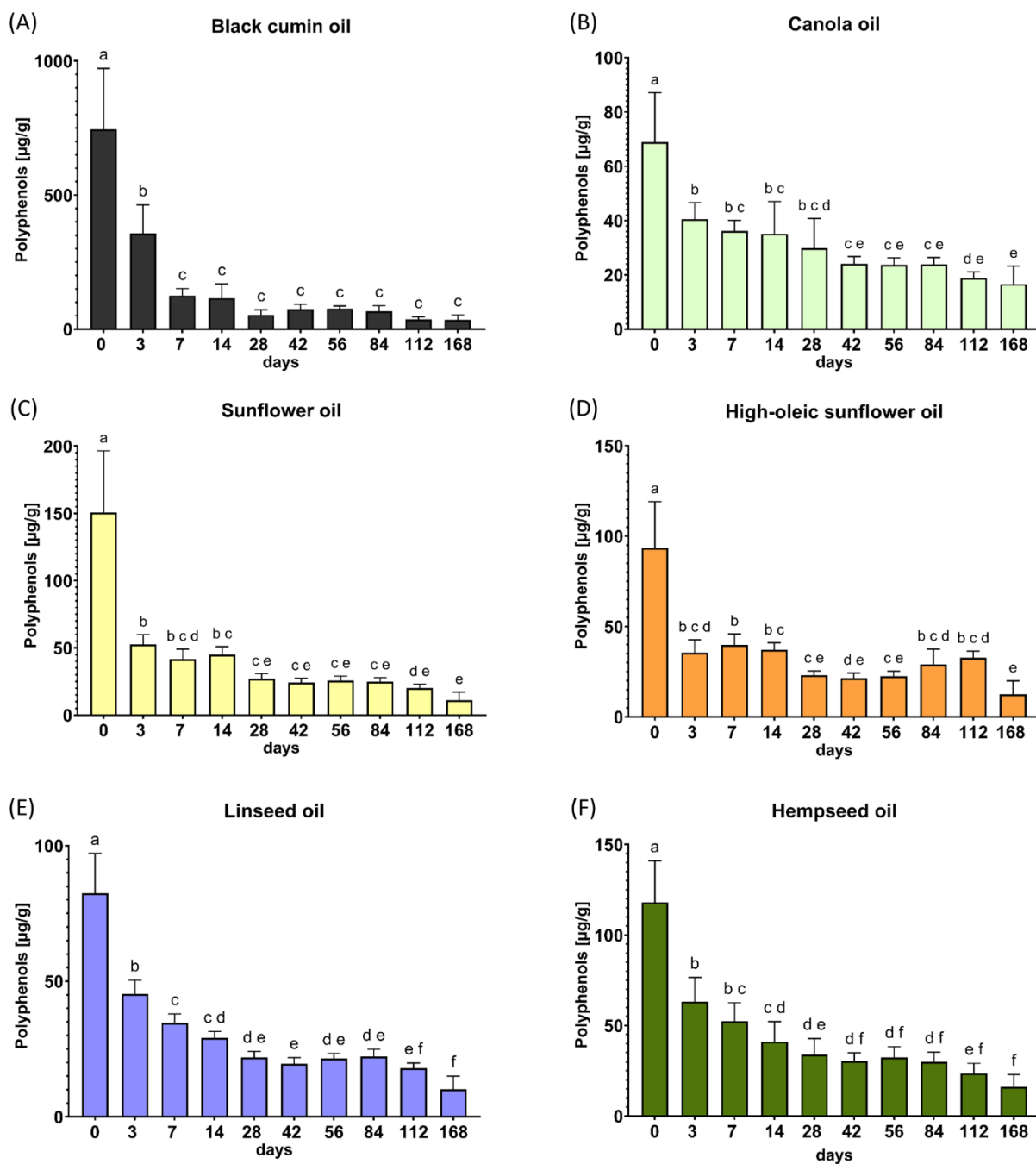


Fig. 2:



8

9

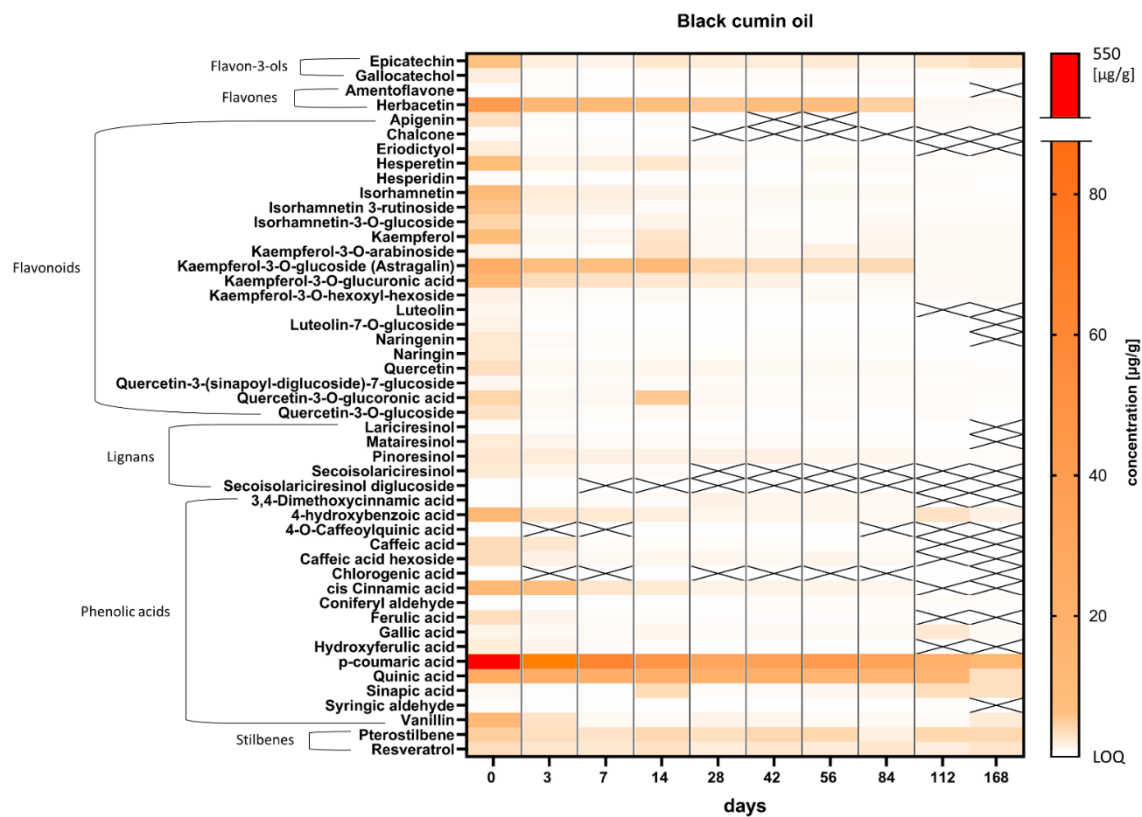
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12

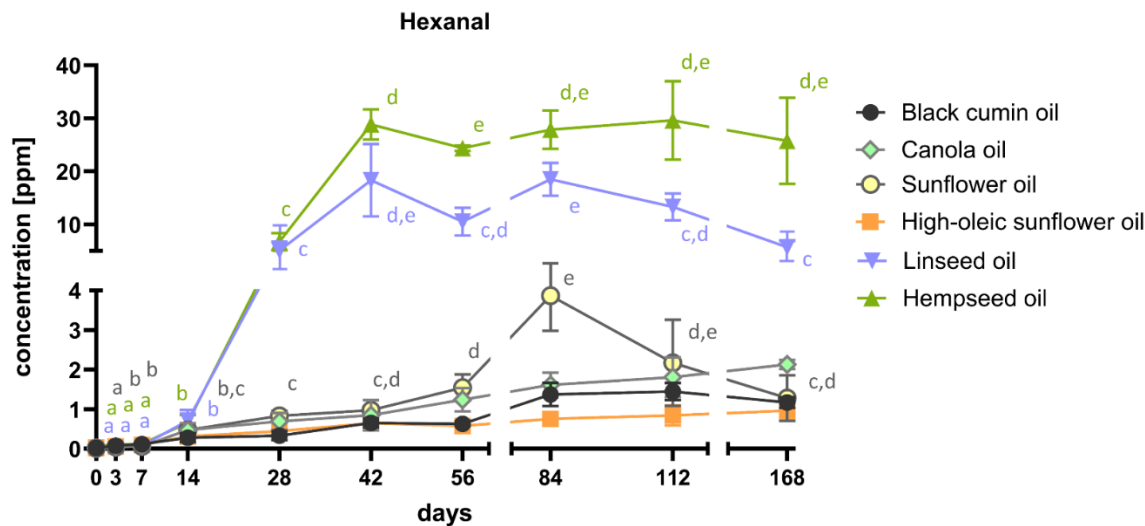


13 Fig. 3:



14

15 Fig. 4:



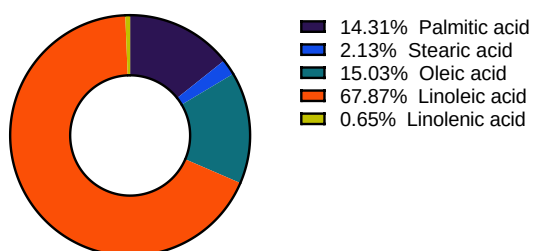
16

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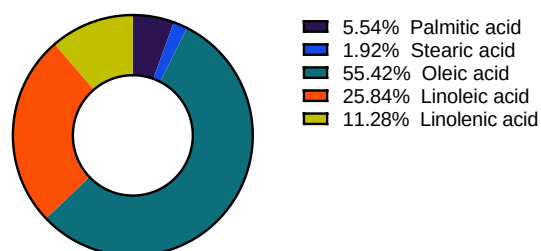
Supplementary Material

**Fig. S1:** Setup of storage study with 54 oil samples (black cumin, canola, sunflower, high-oleic sunflower, linseed and hempseed oil from three different suppliers in triplicates) in PET bottles stored at room temperature for 12 h under cold fluorescent light per day for six months.

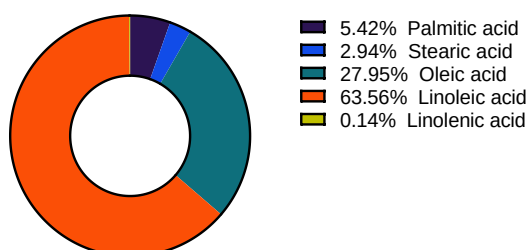
(A) **Black cumin oil**



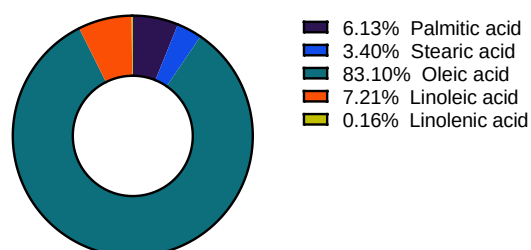
(B) **Canola oil**



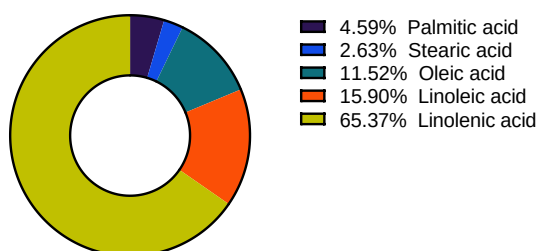
(C) **Sunflower oil**



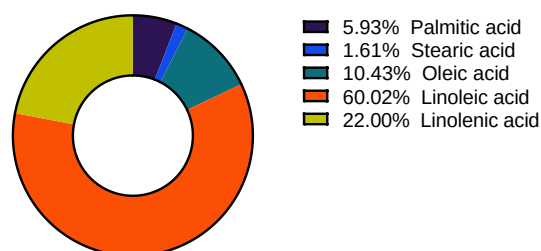
(D) **High-oleic sunflower oil**



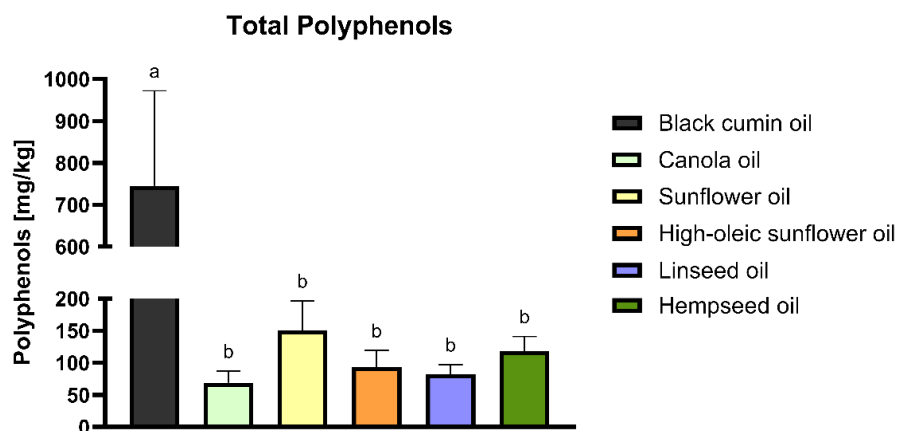
(E) **Linseed oil**



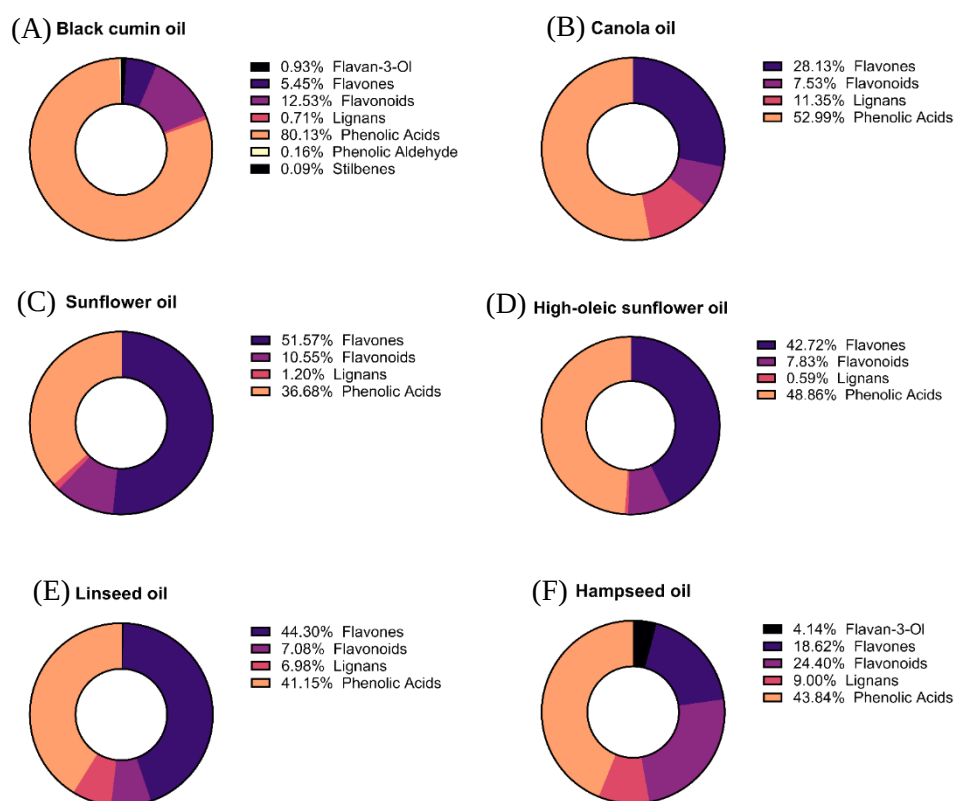
(F) **Hempseed oil**



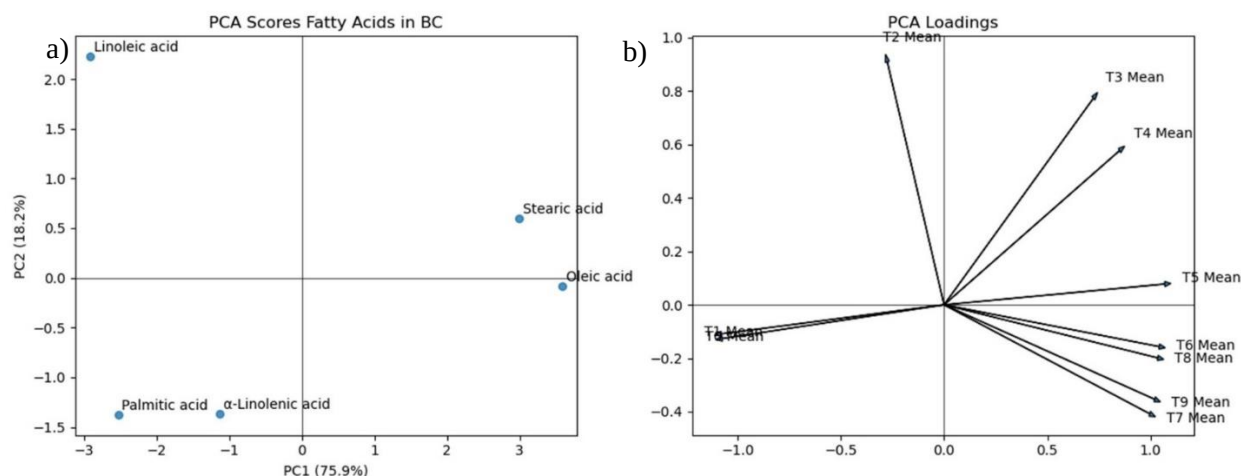
**Fig S2:** Initial relative fatty acid composition [%] (at day 0) of each oil: (A) black cumin, (B) canola, (C) sunflower, (D) high-oleic sunflower, (E) linseed and (F) hempseed oil. Values are shown as mean,  $n \geq 9$ .



**Fig. S3:** Total polyphenol content in mg/kg oil in six cold-pressed oils at day 0 ( $T_0$ ) determined by LC-MS/MS. One-way ANOVA was conducted. Different lowercase letters indicate significant differences ( $p < 0.05$ , ANOVA with Tukey's post hoc test). Values are shown as mean + SD,  $n \geq 9$



**Fig. S4:** Relative composition of polyphenol classes [%] in six cold-pressed oils at  $T_0$  (day 0), averaged across three suppliers. Data obtained via LC-MS/MS classification into phenolic acids, flavonoids, stilbenes, and lignans. Values are shown as relative mean of total polyphenol content,  $n \geq 9$ .



**Figure S5:** a) *Principal Component Analysis (PCA) of fatty acid profiles in black cumini oil (BC). Scores plot showing separation of fatty acids along the first two principal components. b) loadings plot illustrating the contribution of sampling time points (T0–T9, day 0 to 168) to the variance structure. PC1 and PC2 explain 75.9% and 18.2% of the total variance, respectively (cumulative 94.1%).*

**Table S1:** Standard curves used for the fatty acid analysis by GC-FID.  $x$  = concentration [g/kg oil] and  $y$  = area under the curve (AuC) and coefficient of determination ( $R^2$ ).

| Standard          | Formula                | $R^2$ | LOD [ $\mu\text{g/g oil}$ ] | LOQ [ $\mu\text{g/g oil}$ ] |
|-------------------|------------------------|-------|-----------------------------|-----------------------------|
| Methyl palmytate  | $y = 1.7026x - 0.1025$ | 99.86 | 14                          | 43                          |
| Methyl stearate   | $y = 1.2663x - 0.0452$ | 98.67 | 1.82                        | 54                          |
| Methyl oleate     | $y = 1.654x - 0.4769$  | 99.63 | 84                          | 255                         |
| Methyl linoleate  | $y = 1.7386x - 0.0844$ | 99.91 | 13                          | 39                          |
| Methyl linolenate | $y = 1.3398x - 0.0057$ | 99.90 | 1                           | 4                           |

**Table S2:** Transition list used of all analyzed polyphenolic substances separated in classes with its precursor mass and product mass  $m/z$  values for data acquisition of LC-MS/MS analysis (MRM).

| Polyphenol Class | Molecule Name              | Precursor [ $m/z$ ] | Product [ $m/z$ ]        |
|------------------|----------------------------|---------------------|--------------------------|
| Phenolic Acids   | 1,3-Dicaffeoylquinic acid  | 515.0               | 353.0, 191.0, 171.0      |
| Phenolic Acids   | 3,4-Dimethoxycinnamic acid | 207.1               | 207.1, 119.0, 103.0      |
| Phenolic Acids   | 4-hydroxybenzoic acid      | 137.0               | 119.1, 108.0, 93.0, 59.2 |
| Phenolic Acids   | 4-O-Caffeoylquinic acid    | 353.1               | 191.0, 179.0, 173.0      |

| Polyphenol Class | Molecule Name                   | Precursor [m/z] | Product [m/z]       |
|------------------|---------------------------------|-----------------|---------------------|
| Flavones         | Amentoflavone                   | 539.1           | 403.0, 377.1        |
| Flavonoids       | Apigenin                        | 269.0           | 151.0, 117.0, 107.0 |
| Flavonoids       | Astragalin                      | 447.4           | 284.0, 255.0, 227.0 |
| Phenolic Acids   | Caffeic acid                    | 179.0           | 135.0, 107.0, 97.0  |
| Phenolic Acids   | Caffeic acid hexoside           | 341.1           | 179.0, 135.0, 103.0 |
| Flavonoids       | Chalcone                        | 209.1           | 131.0, 103.0        |
| Phenolic Acids   | Chlorogenic acid                | 353.1           | 191.2, 179.0        |
| Phenolic Acids   | cis Cinnamic acid               | 149.1           | 131.0, 103.0        |
| Phenolic Acids   | Coniferyl aldehyde              | 177.1           | 162.0, 81.0         |
| Phenolic Acids   | Coumaric acid hexoside          | 325.1           | 205.0, 145.0, 117.0 |
| Phenolic Acids   | Ellagic acid                    | 303.0           | 257.0, 229.0        |
| Flavan-3-Ol      | Epicatechin                     | 289.1           | 245.1, 125.0, 109.0 |
| Flavonoids       | Eriodictyol                     | 287.1           | 255.0, 151.0, 135.0 |
| Phenolic Acids   | Ferulic acid                    | 195.0           | 177.0, 117.0, 89.0  |
| Phenolic Acids   | Gallic acid                     | 169.0           | 125.0, 96.6, 79.1   |
| Flavan-3-Ol      | Galocatechol                    | 305.1           | 137.0, 125.0        |
| Flavones         | Herbacetin                      | 303.0           | 169.0, 133.0, 121.0 |
| Flavonoids       | Hesperetin                      | 303.3           | 177.0, 153.0        |
| Flavonoids       | Hesperidin                      | 609.2           | 301.1               |
| Phenolic Acids   | Hydroxyferulic acid             | 209.0           | 165.0, 121.0        |
| Phenolic Acids   | Isoferulic acid                 | 193.0           | 178.3, 133.8        |
| Flavonoids       | Isorhamnetin                    | 315.0           | 151.0, 107.0        |
| Flavonoids       | Isorhamnetin 3-rutinoside       | 625.2           | 302.0               |
| Flavonoids       | Isorhamnetin-3-O-glucoside      | 477.1           | 314.0, 271.0, 243.0 |
| Flavonoids       | Kaempferol                      | 285.1           | 257.0, 229.0        |
| Flavonoids       | Kaempferol-3-O-arabinoside      | 417.0           | 284.0, 255.0, 227.0 |
| Flavonoids       | Kaempferol-3-O-glucoside        | 447.1           | 285.0, 247.1        |
| Flavonoids       | Kaempferol-3-O-glucuronic acid  | 461.1           | 285.0, 229.0        |
| Flavonoids       | Kaempferol-3-O-hexoxyl-hexoside | 609.1           | 284.0               |
| Lignans          | Lariciresinol                   | 359.1           | 330.0, 160.1        |
| Flavonoids       | Luteolin                        | 285.0           | 151.0, 133.0, 107.0 |
| Flavonoids       | Luteolin-7-O-glucoside          | 447.1           | 285.0, 151.0        |
| Lignans          | Matairesinol                    | 357.1           | 137.0, 122.0, 83.0  |
| Flavonoids       | Naringenin                      | 271.2           | 151.0, 119.0        |
| Flavonoids       | Naringin                        | 579.2           | 459.0, 271.1, 151.0 |
| Phenolic Acids   | p-coumaric acid                 | 165.0           | 147.0, 119.0, 91.0  |
| Lignans          | Pinoresinol                     | 357.1           | 151.0, 136.0        |
| Phenolic Acids   | Protocatechuic acid             | 153.0           | 109.0, 81.0         |
| Stilbenes        | Pterostilbene                   | 255.1           | 195.0, 169.0        |
| Flavonoids       | Quercetin                       | 301.0           | 151.0, 121.0, 93.0  |



| Polyphenol Class  | Molecule Name                                  | Precursor [m/z] | Product [m/z]       |
|-------------------|------------------------------------------------|-----------------|---------------------|
| Flavonoids        | Quercetin-3-(sinapoyl-diglucoside)-7-glucoside | 625.1           | 463.0, 301.0        |
| Flavonoids        | Quercetin-3-O-glucuronic acid                  | 477.1           | 301.0, 151.0, 107.0 |
| Flavonoids        | Quercetin-3-O-glucoside                        | 463.0           | 301.0, 271.0        |
| Phenolic Acids    | Quinic acid                                    | 191.0           | 93.0, 85.0          |
| Stilbenes         | Resveratrol                                    | 227.0           | 185.0, 143.0        |
| Flavonoids        | Rutin                                          | 609.0           | 300.0, 271.0        |
| Lignans           | Secoisolariciresinol                           | 363.2           | 327.2, 137.1        |
| Lignans           | Secoisolariciresinol diglucoside               | 685.0           | 523.0, 361.0        |
| Phenolic Acids    | Sinapic acid                                   | 223.0           | 163.0, 149.0        |
| Phenolic Acids    | Syringic acid                                  | 197.0           | 120.9, 105.8        |
| Phenolic Acids    | Syringic aldehyde                              | 181.0           | 151.0, 123.0        |
| Phenolic Acids    | Vanillic acid                                  | 167.0           | 152.0, 108.0        |
| Phenolic Aldehyde | Vanillin                                       | 151.0           | 136.0, 92.0         |

**Table S3:** Standard curves from each of the representative standards used for quantification of polyphenolic substances identified with  $x$  = concentration [ $\mu\text{g/g}$  oil] and  $y$  = area under the curve (AuC) by LC-MS/MS with limit of detection (LOD) (signal-to-noise ratio of 3) and a limit of quantification (LOQ) (signal-to-noise ratio of 10) and coefficient of determination ( $R^2$ ).

| Standard              | Formula                | $R^2$ | LOD [ $\mu\text{g/g}$ ] | LOQ [ $\mu\text{g/g}$ ] |
|-----------------------|------------------------|-------|-------------------------|-------------------------|
| Kaempferol            | $y = 6976x - 7692$     | 97.56 | 2.17                    | 7.23                    |
| Quercetin-3-glucoside | $y = 44641x - 95622$   | 98.27 | 1.82                    | 6.07                    |
| Naringenin            | $y = 131240x - 122238$ | 98.73 | 1.56                    | 5.20                    |
| Apigenin              | $y = 185771x - 124518$ | 99.87 | 0.46                    | 1.54                    |
| Epicatechin           | $y = 2059x + 32561$    | 99.04 | 1.61                    | 5.37                    |
| Secoisolariciresinol  | $y = 771x + 545$       | 99.58 | 0.52                    | 1.73                    |
| Caffeic acid          | $y = 96713x + 349596$  | 99.83 | 1.31                    | 4.39                    |
| Ferulic acid          | $y = 190086x - 121795$ | 98.16 | 1.88                    | 6.27                    |

|                       |                        |       |      |      |
|-----------------------|------------------------|-------|------|------|
| p-coumaric acid       | $y = 123484x - 305192$ | 99.13 | 1.29 | 4.29 |
| Gallic acid           | $y = 28929x + 61422$   | 99.64 | 0.82 | 2.74 |
| Vanillic acid         | $y = 3721x - 1749$     | 99.65 | 0.99 | 3.28 |
| 4-hydroxybenzoic acid | $y = 23581x + 116242$  | 99.72 | 0.91 | 3.04 |
| Resveratrol           | $y = 1252x - 870$      | 99.36 | 0.96 | 3.19 |

**Table S4:** Secondary lipid oxidation products used for quantification. Calibration curve equations are shown with coefficient of determination ( $R^2$ ).

| Standard    | Formula                  | $R^2$ |
|-------------|--------------------------|-------|
| Hexanal     | $y = 835429x + 5432321$  | 95.81 |
| 2-pentanone | $y = 3145202x - 4562391$ | 98.41 |
| 2-heptanone | $y = 1337482x + 2163811$ | 98.32 |
| 1-hexanol   | $y = 1377884x + 2764936$ | 91.56 |

**Table S51:** Targeted LC-MS/MS analysis of polyphenols in six cold-pressed oils. Data given as mean  $\pm$  standard deviation (SD) in ppm, n=9.

| Black Cumin Oil  |                            |                  |                  |                  |                 |                 |                 |                 |                 |                 |                 |
|------------------|----------------------------|------------------|------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Polyphenol Class | Substance                  | Day 0            | Day 3            | Day 7            | Day 14          | Day 28          | Day 42          | Day 56          | Day 84          | Day 112         | Day 168         |
| Flavan-3-Ol      | Epicatechin                | 5.64 $\pm$ 1.74  | 1.23 $\pm$ 0.36  | 0.80 $\pm$ 0.19  | 1.90 $\pm$ 0.63 | 1.47 $\pm$ 0.38 | 1.37 $\pm$ 0.32 | 1.72 $\pm$ 0.57 | 0.70 $\pm$ 0.04 | 1.94 $\pm$ 0.15 | 2.55 $\pm$ 1.00 |
|                  |                            | 1.31 $\pm$ 0.28  | 0.20 $\pm$ 0.02  | LOQ              | 0.28 $\pm$ 0.02 | 0.29 $\pm$ 0.02 | 0.29 $\pm$ 0.01 | 0.34 $\pm$ 0.13 | 0.38 $\pm$ 0.02 | 0.31 $\pm$ 0.04 | LOQ             |
| Flavan-3-Ol      | Gallocatechol              | 0.11 $\pm$ 0.03  | 0.22 $\pm$ 0.08  | LOQ              | LOQ             | LOQ             | LOQ             | LOQ             | LOD             | LOD             | LOD             |
| Flavones         | Amentoflavone              | 40.63 $\pm$ 8.12 | 13.22 $\pm$ 3.84 | 11.32 $\pm$ 1.54 | 5.19 $\pm$ 1.87 | 4.38 $\pm$ 0.67 | 6.08 $\pm$ 1.42 | 6.53 $\pm$ 1.15 | 3.73 $\pm$ 1.07 | 0.58 $\pm$ 0.29 | 0.54 $\pm$ 0.22 |
| Flavones         | Herbacetin                 | 2.48 $\pm$ 1.04  | 0.22 $\pm$ 0.11  | 0.11 $\pm$ 0.05  | 0.29 $\pm$ 0.13 | LOQ             | LOD             | LOD             | LOD             | 0.34 $\pm$ 0.06 | LOQ             |
| Flavonoids       | Apigenin                   | 0.29 $\pm$ 0.10  | LOQ              | LOQ              | LOD             | LOD             | LOD             | LOD             | LOD             | LOD             | LOD             |
| Flavonoids       | Chalcone                   | 1.47 $\pm$ 0.39  | 0.28 $\pm$ 0.07  | 0.30 $\pm$ 0.04  | 0.26 $\pm$ 0.01 | 0.24 $\pm$ 0.08 | LOQ             | LOQ             | LOQ             | LOD             | LOD             |
| Flavonoids       | Eriodictyol                | 7.52 $\pm$ 2.01  | 0.93 $\pm$ 0.21  | 1.13 $\pm$ 0.62  | 1.85 $\pm$ 1.02 | 0.63 $\pm$ 0.18 | LOD             | LOQ             | LOQ             | LOD             | LOD             |
| Flavonoids       | Hesperetin                 | 0.28 $\pm$ 0.06  | LOQ              | LOQ              | LOQ             | LOD             | LOD             | LOD             | LOD             | LOD             | LOD             |
| Flavonoids       | Hesperidin                 | 10.92 $\pm$ 3.24 | 1.46 $\pm$ 0.54  | 1.22 $\pm$ 0.34  | 0.96 $\pm$ 0.53 | 0.58 $\pm$ 0.24 | 0.48 $\pm$ 0.11 | 0.42 $\pm$ 0.07 | 0.41 $\pm$ 0.20 | LOD             | LOD             |
| Flavonoids       | Isorhamnetin               | 4.63 $\pm$ 1.34  | 1.13 $\pm$ 0.43  | 0.92 $\pm$ 0.34  | 0.29 $\pm$ 0.03 | 0.27 $\pm$ 0.05 | 0.34 $\pm$ 0.09 | LOQ             | LOQ             | LOD             | LOD             |
| Flavonoids       | 3-rutinoside               | 3.38 $\pm$ 0.46  | 0.47 $\pm$ 0.16  | 0.23 $\pm$ 0.02  | 0.89 $\pm$ 0.16 | 0.44 $\pm$ 0.15 | LOQ             | LOQ             | 0.50 $\pm$ 0.27 | 0.34 $\pm$ 0.01 | 0.32 $\pm$ 0.11 |
| Flavonoids       | Isorhamnetin-3-O-glucoside | 9.71 $\pm$ 1.33  | 0.63 $\pm$ 0.14  | 0.74 $\pm$ 0.20  | 2.01 $\pm$ 1.37 | 0.53 $\pm$ 0.18 | 0.58 $\pm$ 0.15 | LOQ             | 0.74 $\pm$ 0.22 | 0.48 $\pm$ 0.11 | 0.40 $\pm$ 0.07 |
| Flavonoids       | Kaempferol                 |                  |                  |                  |                 |                 |                 |                 |                 |                 |                 |

|                          |                                                |                |                |              |               |              |              |               |              |              |              |
|--------------------------|------------------------------------------------|----------------|----------------|--------------|---------------|--------------|--------------|---------------|--------------|--------------|--------------|
| <b>Flavonoids</b>        | Kaempferol-3-O-arabinoside                     | 0.89 ± 0.18    | 0.38 ± 0.14    | 0.23 ± 0.07  | 2.23 ± 0.06   | 0.53 ± 0.22  | 0.54 ± 0.22  | 1.13 ± 0.38   | 0.82 ± 0.39  | 0.46 ± 0.09  | 0.43 ± 0.19  |
| <b>Flavonoids</b>        | Kaempferol-3-O-glucoside (Astragalin)          | 22.60 ± 3.60   | 6.65 ± 1.82    | 6.90 ± 1.58  | 12.40 ± 6.24  | 3.12 ± 1.07  | 2.67 ± 0.86  | 2.62 ± 0.68   | 2.93 ± 1.37  | 0.46 ± 0.17  | 0.41 ± 0.24  |
| <b>Flavonoids</b>        | Kaempferol-3-O-glucuronic acid                 | 13.76 ± 3.68   | 2.74 ± 0.88    | 2.25 ± 0.60  | 1.81 ± 0.79   | 1.48 ± 0.60  | 1.05 ± 0.36  | 0.19 ± 0.07   | 0.46 ± 0.04  | 0.51 ± 0.19  | 0.46 ± 0.15  |
| <b>Flavonoids</b>        | Kaempferol-3-O-hexoxyl-hexoside                | 1.01 ± 0.27    | 0.37 ± 0.11    | 0.17 ± 0.04  | 0.42 ± 0.16   | 0.20 ± 0.03  | 0.17 ± 0.02  | 0.46 ± 0.01   | LOQ          | 0.38 ± 0.02  | 0.46 ± 0.19  |
| <b>Flavonoids</b>        | Luteolin                                       | 0.65 ± 0.37    | 0.18 ± 0.03    | LOQ          | LOQ           | LOD          | LOD          | LOD           | LOD          | LOD          | LOD          |
| <b>Flavonoids</b>        | Luteolin-7-O-glucoside                         | 0.88 ± 0.28    | LOD            | LOD          | LOD           | LOD          | LOD          | LOD           | LOD          | LOD          | LOD          |
| <b>Flavonoids</b>        | Naringenin                                     | 1.74 ± 0.26    | 0.34 ± 0.08    | 0.16 ± 0.03  | 0.20 ± 0.02   | 0.15 ± 0.03  | 0.16 ± 0.06  | LOD           | LOD          | LOD          | LOD          |
| <b>Flavonoids</b>        | Naringin                                       | 1.61 ± 0.45    | 0.20 ± 0.10    | 0.06 ± 0.02  | 0.23 ± 0.09   | 0.16 ± 0.01  | 0.17 ± 0.02  | 0.19 ± 0.06   | LOD          | LOD          | LOD          |
| <b>Flavonoids</b>        | Quercetin                                      | 2.51 ± 0.35    | 0.51 ± 0.10    | 0.42 ± 0.10  | 0.70 ± 0.30   | 0.65 ± 0.20  | 0.43 ± 0.05  | 0.44 ± 0.11   | 0.44 ± 0.08  | 0.24 ± 0.09  | LOD          |
| <b>Flavonoids</b>        | quercetin-3-(sinapoyl-diglucoside)-7-glucoside | 0.63 ± 0.18    | 0.32 ± 0.05    | 0.33 ± 0.05  | 0.32 ± 0.03   | 0.35 ± 0.09  | 0.23 ± 0.04  | 0.32 ± 0.10   | 0.26 ± 0.03  | 0.22 ± 0.02  | 0.29 ± 0.04  |
| <b>Flavonoids</b>        | Quercetin-3-O-glucuronic acid                  | 3.28 ± 0.96    | 0.58 ± 0.17    | 0.48 ± 0.05  | 4.34 ± 0.82   | 0.48 ± 0.13  | 0.28 ± 0.03  | LOQ           | 0.25 ± 0.03  | 0.28 ± 0.11  | LOQ          |
| <b>Flavonoids</b>        | Quercetin-3-O-glucoside                        | 2.22 ± 0.45    | 0.32 ± 0.05    | 0.21 ± 0.07  | 0.44 ± 0.11   | 0.31 ± 0.11  | LOQ          | 0.27 ± 0.07   | LOD          | 0.32 ± 0.07  | LOD          |
| <b>Lignans</b>           | Lariciresinol                                  | 0.27 ± 0.09    | 0.18 ± 0.01    | 0.08 ± 0.03  | LOD           | LOD          | LOD          | LOD           | LOD          | LOD          | LOD          |
| <b>Lignans</b>           | Matairesinol                                   | 1.51 ± 0.19    | 0.72 ± 0.28    | 0.28 ± 0.06  | 0.35 ± 0.13   | 0.39 ± 0.13  | 0.30 ± 0.08  | 0.20 ± 0.07   | 0.23 ± 0.07  | LOD          | LOD          |
| <b>Lignans</b>           | Pinoresinol                                    | 1.85 ± 0.14    | 1.43 ± 0.41    | 1.07 ± 0.56  | 1.09 ± 0.60   | 1.01 ± 0.39  | 1.12 ± 0.52  | 0.64 ± 0.18   | 0.68 ± 0.38  | 0.29 ± 0.08  | 0.18 ± 0.08  |
| <b>Lignans</b>           | Secoisolariciresinol                           | 1.59 ± 0.46    | 0.66 ± 0.28    | 0.29 ± 0.10  | 0.35 ± 0.03   | LOD          | LOD          | LOD           | LOD          | LOD          | LOD          |
| <b>Lignans</b>           | Secoisolariciresinol diglucoside               | LOQ            | LOQ            | LOD          | LOD           | LOD          | LOD          | LOD           | LOD          | LOD          | LOD          |
| <b>Phenolic Acids</b>    | 3,4-Dimethoxycinnamic acid                     | LOQ            | LOQ            | 0.26 ± 0.05  | 0.32 ± 0.04   | 1.09 ± 0.26  | 0.76 ± 0.06  | 0.64 ± 0.03   | 0.49 ± 0.06  | LOD          | LOD          |
| <b>Phenolic Acids</b>    | 4-hydroxybenzoic acid                          | 13.07 ± 1.18   | 2.29 ± 0.58    | 1.73 ± 0.37  | 1.26 ± 0.59   | 0.75 ± 0.16  | 0.79 ± 0.17  | 0.64 ± 0.19   | 0.58 ± 0.12  | 2.30 ± 0.18  | 1.07 ± 0.63  |
| <b>Phenolic Acids</b>    | 4-O-Caffeoylquinic acid                        | LOQ            | LOQ            | LOQ          | LOQ           | LOD          | LOQ          | LOD           | LOD          | LOD          | LOD          |
| <b>Phenolic Acids</b>    | Caffeic acid                                   | 2.74 ± 0.58    | 1.88 ± 1.05    | LOQ          | LOQ           | LOQ          | LOQ          | LOQ           | LOQ          | LOD          | LOD          |
| <b>Phenolic Acids</b>    | Caffeic acid hexoside                          | 2.73 ± 0.13    | 1.03 ± 0.26    | 0.41 ± 0.07  | 0.67 ± 0.34   | 0.70 ± 0.26  | 0.45 ± 0.13  | 0.78 ± 0.30   | 0.49 ± 0.16  | LOD          | LOD          |
| <b>Phenolic Acids</b>    | Chlorogenic acid                               | LOQ            | LOQ            | LOQ          | LOQ           | LOQ          | LOQ          | LOD           | LOQ          | LOQ          | LOD          |
| <b>Phenolic Acids</b>    | cis Cinnamic acid                              | 11.31 ± 1.95   | 6.30 ± 2.21    | 1.96 ± 0.47  | 1.61 ± 0.80   | 0.94 ± 0.26  | 0.88 ± 0.22  | 0.87 ± 0.19   | 0.73 ± 0.23  | LOD          | LOD          |
| <b>Phenolic Acids</b>    | Coniferyl aldehyde                             | 0.04 ± 0.00    | 0.02 ± 0.00    | 0.07 ± 0.04  | 0.10 ± 0.06   | 0.15 ± 0.05  | 0.24 ± 0.11  | 0.28 ± 0.13   | LOQ          | LOQ          | LOQ          |
| <b>Phenolic Acids</b>    | Ferulic acid                                   | 2.53 ± 0.49    | 0.83 ± 0.15    | 0.24 ± 0.05  | LOQ           | LOQ          | LOQ          | LOQ           | LOQ          | LOD          | LOD          |
| <b>Phenolic Acids</b>    | Gallic acid                                    | 0.86 ± 0.19    | 0.37 ± 0.15    | 0.38 ± 0.08  | 0.63 ± 0.31   | 0.24 ± 0.08  | 0.54 ± 0.19  | 0.46 ± 0.21   | 0.43 ± 0.12  | 1.77 ± 0.44  | LOQ          |
| <b>Phenolic Acids</b>    | Hydroxyferulic acid                            | 1.46 ± 0.72    | 0.80 ± 0.22    | 0.26 ± 0.07  | LOQ           | LOQ          | LOQ          | LOQ           | LOD          | LOD          | LOD          |
| <b>Phenolic Acids</b>    | p-coumaric acid                                | 538.20 ± 68.93 | 248.12 ± 66.03 | 63.40 ± 9.22 | 46.57 ± 19.22 | 29.86 ± 7.15 | 34.08 ± 7.72 | 41.69 ± 10.37 | 33.64 ± 8.24 | 22.02 ± 8.52 | 13.22 ± 8.78 |
| <b>Phenolic Acids</b>    | Quinic acid                                    | 25.32 ± 1.23   | 23.84 ± 1.33   | 23.55 ± 1.07 | 21.08 ± 7.08  | 20.20 ± 1.49 | 19.21 ± 1.52 | 16.64 ± 0.89  | 14.12 ± 0.91 | 15.71 ± 5.49 | 2.42 ± 1.77  |
| <b>Phenolic Acids</b>    | Sinapic acid                                   | 0.48 ± 0.10    | 0.11 ± 0.01    | 0.07 ± 0.02  | 2.85 ± 1.93   | 0.31 ± 0.10  | 0.39 ± 0.22  | 0.62 ± 0.28   | 0.86 ± 0.45  | 2.65 ± 0.18  | 2.41 ± 1.77  |
| <b>Phenolic Acids</b>    | Syringic aldehyde                              | 0.08 ± 0.01    | 0.04 ± 0.01    | 0.01 ± 0.00  | 0.04 ± 0.00   | LOD          | LOD          | LOD           | LOD          | 0.13 ± 0.07  | LOD          |
| <b>Phenolic Aldehyde</b> | Vanillin                                       | 12.25 ± 1.33   | 2.18 ± 1.09    | 0.43 ± 0.11  | 0.38 ± 0.14   | 0.83 ± 0.02  | 0.76 ± 0.07  | 0.34 ± 0.05   | 0.45 ± 0.07  | 0.31 ± 0.12  | 1.50 ± 0.81  |
| <b>Stilbenes</b>         | Pterostilbene                                  | 3.81 ± 0.91    | 2.50 ± 0.79    | 2.16 ± 0.23  | 3.07 ± 0.45   | 2.46 ± 0.41  | 3.06 ± 0.39  | 3.08 ± 0.84   | 1.19 ± 0.06  | 3.12 ± 0.95  | 3.00 ± 0.59  |

|                |                            |              |             |             |             |             |             |             |             |             |             |
|----------------|----------------------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Stilbenes      | Resveratrol                | 2.63 ± 0.70  | 1.96 ± 0.74 | 1.79 ± 0.65 | 2.29 ± 0.35 | 1.41 ± 0.52 | 1.83 ± 0.67 | 1.54 ± 0.09 | 1.85 ± 0.21 | 1.31 ± 0.23 | 1.99 ± 0.43 |
|                | 5-Ethyl-2(5)-furanone      | 0.30 ± 0.09  | 5.08 ± 1.68 | 0.03 ± 0.01 |             |             |             |             |             |             |             |
| Canola Oil     |                            |              |             |             |             |             |             |             |             |             |             |
| Flavones       | Amentoflavone              | 0.41± 0.04   | 0.60± 0.23  | 0.31± 0.12  | 0.39± 0.14  | 0.20± 0.02  | 0.44± 0.08  | 0.47± 0.14  | 0.42± 0.08  | LOD         | LOQ         |
|                |                            | 19.11± 7.09  | 6.32± 2.27  | 4.69± 1.21  | 3.60± 1.06  | 2.85± 1.73  | 2.61± 0.42  | 3.17± 0.66  | 2.56± 0.21  | 1.57± 0.30  | 1.41± 0.51  |
| Flavones       | Herbacetin                 | LOQ          | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
| Flavonoids     | Chalcone                   | 0.06± 0.01   | 0.05± 0.01  | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                |                            | 0.57± 0.16   | LOQ         | 0.45± 0.05  | 0.49± 0.17  | LOQ         | LOQ         | LOQ         | 0.53± 0.10  | LOD         | LOD         |
| Flavonoids     | Eriodictyol                | 1.43± 0.39   | 0.18± 0.07  | 0.22± 0.05  | 0.63± 0.18  | 0.46± 0.28  | 0.17± 0.00  | 0.09± 0.03  | 0.18± 0.00  | 0.18± 0.02  | LOD         |
|                |                            | LOQ          | 0.17± 0.02  | 0.18± 0.02  | LOQ         | 0.26± 0.13  | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Flavonoids     | Hesperidin                 | LOQ          | 0.14± 0.05  | 0.17± 0.05  | 0.18± 0.02  | 0.25± 0.14  | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
|                |                            | LOQ          | 0.05        | 0.05        | 0.02        | 0.14        | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Flavonoids     | Isorhamnetin               | LOQ          | 1.08± 0.13  | 0.29± 0.03  | 0.38± 0.04  | 0.29± 0.09  | 0.62± 0.05  | 0.29± 0.05  | LOQ         | LOD         | LOQ         |
|                |                            | LOQ          | 1.14± 0.25  | 0.48± 0.05  | 0.36± 0.10  | 0.57± 0.19  | 0.58± 0.33  | LOQ         | LOQ         | 0.33± 0.08  | 0.43± 0.13  |
| Flavonoids     | Kaempferol                 | LOQ          | 0.22± 0.04  | LOQ         | LOQ         | 0.42± 0.05  | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Flavonoids     | Naringenin                 | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         |
| Flavonoids     | Quercetin                  | 0.52± 0.10   | 0.26± 0.09  | 0.48± 0.11  | LOQ         | 0.76± 0.34  | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Lignans        | Lariciresinol              | 4.97± 1.93   | 3.45± 1.59  | LOQ         | LOQ         | 1.02± 0.98  | 0.99± 0.47  | 0.56± 0.32  | 1.05± 0.59  | LOQ         | LOQ         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
| Lignans        | Matairesinol               | 1.59± 0.33   | 0.53± 0.17  | 0.43± 0.14  | 0.83± 0.26  | 0.94± 0.60  | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
| Lignans        | Pinoresinol                | 1.05± 0.14   | 0.33± 0.07  | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
| Lignans        | Secoisolariciresinol       | LOQ          | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
| Phenolic Acids | 3,4-Dimethoxycinnamic acid | 0.21± 0.05   | 0.25± 0.03  | 0.31± 0.03  | 0.67± 0.18  | 1.51± 0.94  | 0.73± 0.08  | 0.66± 0.03  | 0.69± 0.04  | LOQ         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         |
| Phenolic Acids | hydroxybenzoic acid        | 0.74± 0.17   | LOQ         | LOQ         | 0.69± 0.18  | 0.51± 0.27  | LOQ         | 0.24± 0.07  | LOQ         | LOQ         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         |
| Phenolic Acids | 4-O-Caffeoylquinic acid    | 0.34± 0.02   | 0.38± 0.05  | 0.39± 0.05  | 0.49± 0.15  | 1.01± 0.23  | 0.30± 0.09  | 0.40± 0.14  | 0.39± 0.11  | LOQ         | LOQ         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Phenolic Acids | Caffeic acid               | 0.24± 0.01   | 0.25± 0.02  | 0.18± 0.05  | 0.63± 0.15  | 0.53± 0.11  | LOQ         | LOQ         | 0.48± 0.08  | 0.39± 0.13  | 0.38± 0.17  |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Phenolic Acids | Caffeic acid hexoside      | 0.77± 0.12   | 0.37± 0.06  | 0.22± 0.03  | 0.54± 0.20  | 0.51± 0.30  | 0.03± 0.01  | 0.10± 0.03  | 0.12± 0.03  | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
| Phenolic Acids | Chlorogenic acid           | 0.22± 0.11   | 0.03± 0.00  | 0.05± 0.01  | 0.08± 0.03  | 0.23± 0.02  | LOD         | LOD         | LOD         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
| Phenolic Acids | cis Cinnamic acid          | 0.53± 0.16   | 0.08± 0.03  | 0.03± 0.00  | 0.37± 0.14  | 0.10± 0.08  | LOD         | LOD         | LOD         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
| Phenolic Acids | Coumaric acid hexoside     | LOQ          | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
| Phenolic Acids | Ellagic acid               | 0.94± 0.45   | 0.14± 0.03  | 0.37± 0.05  | 0.42± 0.12  | 0.33± 0.12  | 0.19± 0.07  | 0.22± 0.04  | 0.19± 0.02  | 0.15± 0.04  | 0.47± 0.17  |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Phenolic Acids | Ferulic acid               | 2.07± 0.66   | 0.53± 0.17  | 0.42± 0.09  | 1.48± 0.52  | 0.56± 0.34  | LOQ         | LOQ         | 0.18± 0.01  | 0.14± 0.03  | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Phenolic Acids | p-coumaric acid            | 3.42± 1.00   | 1.25± 0.21  | 1.43± 0.11  | 1.30± 0.48  | 1.26± 0.59  | 0.61± 0.16  | 0.50± 0.18  | 0.79± 0.19  | 0.34± 0.08  | 0.28± 0.15  |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Phenolic Acids | Quinic acid                | 25.64± 4.74  | 23.85± 0.91 | 23.93± 1.32 | 18.27± 6.86 | 12.54± 1.79 | 16.11± 0.87 | 15.13± 0.58 | 14.34± 0.72 | 13.24± 1.08 | 10.75± 4.61 |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Phenolic Acids | Sinapic acid               | 0.39± 0.04   | 0.13± 0.05  | 0.09± 0.03  | 0.87± 0.07  | 0.17± 0.16  | LOD         | LOD         | LOD         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Phenolic Acids | Syringic aldehyde          | 1.07± 0.12   | 0.32± 0.03  | 0.30± 0.05  | 0.31± 0.08  | 0.91± 0.68  | 0.22± 0.02  | 0.26± 0.07  | 0.25± 0.04  | 0.12± 0.00  | 0.37± 0.09  |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| Sunflower Oil  |                            |              |             |             |             |             |             |             |             |             |             |
| Flavones       | Amentoflavone              | LOQ          | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
| Flavones       | Herbacetin                 | 77.61± 27.74 | 18.44± 3.86 | 9.41± 3.69  | 6.65± 2.54  | 4.10± 1.16  | 4.12± 0.88  | 3.01± 0.94  | 3.32± 0.70  | 2.88± 1.23  | 1.42± 0.73  |
|                |                            | LOQ          | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |



|                |                                       |            |           |           |           |           |           |           |           |           |           |
|----------------|---------------------------------------|------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Flavonoids     | Apigenin                              | LOQ        | LOQ       | LOQ       | LOQ       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       |
| Flavonoids     | Chalcone                              | LOQ        | LOQ       | LOQ       | LOQ       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       |
| Flavonoids     | Eriodictyol                           | 0.99±      | LOQ       | 0.35±     | LOQ       | LOQ       | LOQ       | LOQ       | LOD       | LOQ       | 0.20±     |
|                |                                       | 0.21       |           | 0.06      |           |           |           |           |           |           | 0.13      |
| Flavonoids     | Hesperetin                            | 3.83±      | 0.43±     | 0.31±     | LOQ       | 0.23±     | LOQ       | 0.33±     | 0.40±     | LOQ       | 0.81±     |
|                |                                       | 1.24       | 0.08      | 0.08      |           | 0.06      |           | 0.09      | 0.08      |           | 0.29      |
| Flavonoids     | Isorhamnetin                          | 0.59±      | 0.27±     | 0.35±     | 0.19±     | 0.22±     | 0.29±     | LOQ       | LOQ       | LOQ       | LOQ       |
|                |                                       | 0.16       | 0.01      | 0.01      | 0.05      | 0.05      | 0.07      |           |           |           |           |
| Flavonoids     | Isorhamnetin 3-rutinoside             | 1.17±      | 0.29±     | 0.21±     | 0.26±     | 0.38±     | LOQ       | 0.25±     | 0.32±     | LOQ       | LOQ       |
|                |                                       | 0.23       | 0.10      | 0.08      | 0.05      | 0.08      |           | 0.04      | 0.07      |           |           |
| Flavonoids     | Isorhamnetin-3-O-glucoside            | 1.43±      | 0.13±     | 0.36±     | 0.25±     | 0.34±     | 0.22±     | 0.30±     | 0.20±     | 0.35±     | 0.14±     |
|                |                                       | 0.22       | 0.01      | 0.05      | 0.07      | 0.10      | 0.02      | 0.10      | 0.05      | 0.03      | 0.02      |
| Flavonoids     | Kaempferol                            | 1.35±      | 0.23±     | 0.35±     | LOQ       | 0.44±     | 0.32±     | 0.36±     | 0.32±     | 0.27±     | LOQ       |
|                |                                       | 0.31       | 0.01      | 0.08      |           | 0.12      | 0.03      | 0.08      | 0.08      | 0.08      |           |
| Flavonoids     | Kaempferol-3-O-glucoside (Astragalin) | 1.78±      | 0.41±     | 0.34±     | LOQ       | 0.41±     | 0.31±     | 0.28±     | 0.28±     | LOQ       | LOQ       |
|                |                                       | 0.20       | 0.02      | 0.08      |           | 0.06      | 0.11      | 0.05      | 0.02      |           |           |
| Flavonoids     | Kaempferol-3-O-glucuronic acid        | 0.65±      | 0.44±     | 0.48±     | 0.48±     | 0.26±     | 0.31±     | 0.18±     | 0.48±     | 0.37±     | 0.50±     |
|                |                                       | 0.14       | 0.12      | 0.17      | 0.14      | 0.09      | 0.08      | 0.07      | 0.12      | 0.02      | 0.21      |
| Flavonoids     | Luteolin                              | 0.16±      | 0.06±     | 0.05±     | 0.06±     | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       |
|                |                                       | 0.04       | 0.01      | 0.01      | 0.01      |           |           |           |           |           |           |
| Flavonoids     | Luteolin-7-O-glucoside                | 0.26±      | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       |
| Flavonoids     | Naringenin                            | LOQ        | LOQ       | LOQ       | LOQ       | LOQ       | LOQ       | LOQ       | LOQ       | LOQ       | LOQ       |
| Flavonoids     | Quercetin                             | 1.04±      | 0.15±     | 0.23±     | 0.18±     | 0.33±     | 0.42±     | LOQ       | LOQ       | LOD       | LOQ       |
|                |                                       | 0.28       | 0.02      | 0.03      | 0.04      | 0.04      | 0.05      |           |           |           |           |
| Flavonoids     | Quercetin-3-O-glucuronic acid         | 0.79±      | 0.13±     | 0.16±     | 0.18±     | 0.17±     | 0.17±     | 0.33±     | 0.24±     | 0.32±     | 0.27±     |
|                |                                       | 0.25       | 0.01      | 0.02      | 0.05      | 0.04      | 0.02      | 0.01      | 0.03      | 0.08      | 0.03      |
| Flavonoids     | Quercetin-3-O-glucoside               | 1.39±      | 0.22±     | 0.31±     | 0.30±     | 0.19±     | 0.27±     | 0.37±     | 0.14±     | 0.24±     | 0.13±     |
|                |                                       | 0.15       | 0.02      | 0.02      | 0.06      | 0.05      | 0.11      | 0.12      | 0.00      | 0.02      | 0.05      |
| Flavones       | Amentoflavone                         | LOQ        | LOQ       | LOQ       | LOQ       | LOQ       | LOD       | LOD       | LOD       | LOD       | LOD       |
| Flavones       | Herbacetin                            | 77.61±     | 18.44±    | 9.41±     | 6.65±     | 4.10±     | 4.12±     | 3.01±     | 3.32±     | 2.88±     | 1.42±     |
|                |                                       | 27.74      | 3.86      | 3.69      | 2.54      | 1.16      | 0.88      | 0.94      | 0.70      | 1.23      | 0.73      |
| Flavonoids     | Apigenin                              | 0.18±      | LOQ       | LOQ       | LOQ       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       |
|                |                                       | 0.05       |           |           |           |           |           |           |           |           |           |
| Flavonoids     | Chalcone                              | 0.11±      | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       |
| Lignans        | Matairesinol                          | 0.75±0.18  | 0.59±0.33 | LOD       | 0.18±0.07 | 0.34±0.15 | 0.29±0.08 | 0.47±0.22 | 0.33±0.18 | LOQ       | LOD       |
| Lignans        | Pinoresinol                           | 0.62±0.16  | 0.13±0.05 | LOD       | LOQ       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       |
| Lignans        | Secoisolariciresinol                  | 0.44±0.16  | LOQ       | LOQ       | LOQ       | LOQ       | LOQ       | LOD       | LOD       | LOD       | LOD       |
| Phenolic Acids | 1,3-Dicaffeoylquinic acid             | 0.29±0.03  | LOQ       | LOQ       | LOQ       | LOQ       | LOQ       | LOD       | LOD       | LOD       | LOD       |
| Phenolic Acids | 3,4-Dimethoxycinnamic acid            | 0.11±0.02  | 0.23±0.03 | 0.31±0.04 | 0.25±0.03 | 0.86±0.04 | 0.76±0.05 | 0.56±0.05 | 0.59±0.07 | 0.21±0.02 | LOD       |
| Phenolic Acids | 4-hydroxybenzoic acid                 | 0.54±0.12  | 0.31±0.11 | 0.27±0.01 | 0.26±0.02 | 0.24±0.07 | 0.23±0.09 | 0.33±0.07 | 0.29±0.02 | 0.39±0.09 | 0.26±0.03 |
| Phenolic Acids | 4-O-Caffeoylquinic acid               |            |           |           | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       | LOD       |
| Phenolic Acids | Caffeic acid                          | 0.04±0.00  | 0.02±0.00 | 0.03±0.00 |           |           | LOQ       | LOQ       | LOQ       | LOD       | LOD       |
|                |                                       | 1.05±0.21  | 0.17±0.05 | 0.12±0.04 | 0.13±0.02 | 0.18±0.05 |           |           |           |           |           |
| Phenolic Acids | Caffeic acid hexoside                 | 2.60±0.86  | 0.58±0.16 | 0.41±0.16 | 0.19±0.08 | 0.42±0.09 | 0.01±0.00 | 0.42±0.13 | 0.39±0.11 | 0.18±0.09 | 0.13±0.07 |
| Phenolic Acids | Chlorogenic acid                      | 2.92±1.14  | 0.26±0.15 | 0.23±0.13 | LOQ       | LOQ       | LOQ       | LOQ       | LOQ       | LOD       | LOD       |
| Phenolic Acids | cis Cinnamic acid                     | 2.15±0.76  | 0.20±0.08 | 0.21±0.13 | 0.11±0.05 | 0.17±0.04 | 0.11±0.05 | LOQ       | LOQ       | LOQ       | LOQ       |
| Phenolic Acids | Ellagic acid                          | 16.65±6.51 | 3.13±0.44 | 1.69±0.73 | 0.69±0.29 | 0.94±0.17 | 0.71±0.34 | 0.30±0.09 | 0.55±0.08 | 0.49±0.10 | 0.45±0.28 |

|                         |                                       |             |            |            |            |            |            |            |            |           |           |
|-------------------------|---------------------------------------|-------------|------------|------------|------------|------------|------------|------------|------------|-----------|-----------|
| <b>Phenolic Acids</b>   | Ferulic acid                          | 0.80±0.06   | 0.12±0.02  | 0.13±0.02  | 0.14±0.03  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ       | LOD       |
| <b>Phenolic Acids</b>   | p-coumaric acid                       | 7.50±2.17   | 1.35±0.37  | 1.48±0.54  | 1.17±0.24  | 0.71±0.20  | 0.72±0.14  | 0.51±0.21  | 1.46±0.41  | 0.48±0.12 | 0.36±0.25 |
| <b>Phenolic Acids</b>   | Quinic acid                           | 20.63±1.98  | 23.88±1.07 | 23.15±1.20 | 32.03±1.96 | 15.30±0.86 | 13.59±0.77 | 12.59±0.79 | 10.51±0.47 | 9.47±0.42 | 4.54±3.11 |
| <b>HO Sunflower Oil</b> |                                       |             |            |            |            |            |            |            |            |           |           |
| <b>Flavones</b>         | Herbacetin                            | 39.76±14.50 | 3.92±0.88  | 7.56±2.77  | 1.32±0.29  | 2.59±0.70  | 2.62±0.75  | 2.60±0.92  | 2.22±1.15  | 1.83±0.36 | 1.36±0.78 |
| <b>Flavonoids</b>       | Apigenin                              | LOQ         | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD        | LOD        | LOD       | LOD       |
| <b>Flavonoids</b>       | Chalcone                              | LOD         | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       | LOD       |
| <b>Flavonoids</b>       | Eriodictyol                           | 0.69±0.15   | 0.20±0.04  | 0.35±0.09  | 0.18±0.01  | 0.20±0.07  | LOQ        | LOD        | 0.49±0.08  | LOQ       | LOQ       |
| <b>Flavonoids</b>       | Hesperetin                            | 2.35±0.62   | 0.32±0.13  | 0.37±0.12  | LOQ        | LOQ        | LOQ        | LOQ        | 0.38±0.21  | LOQ       | LOD       |
| <b>Flavonoids</b>       | Hesperidin                            | 0.33±0.03   | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD        | LOD       | LOD       |
| <b>Flavonoids</b>       | Isorhamnetin                          | 0.79±0.31   | 0.25±0.02  | 0.38±0.14  | 0.38±0.03  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ       | LOQ       |
| <b>Flavonoids</b>       | Isorhamnetin-3-O-glucoside            | 0.39±0.18   | 0.36±0.03  | 0.21±0.02  | 0.37±0.08  | LOQ        | 0.36±0.02  | LOQ        | LOQ        | LOQ       | LOQ       |
| <b>Flavonoids</b>       | Kaempferol                            | 1.15±0.29   | LOQ        | 0.46±0.05  | 0.42±0.04  | 0.29±0.04  | LOQ        | 0.30±0.02  | 0.45±0.16  | 0.48±0.03 | LOQ       |
| <b>Flavonoids</b>       | Kaempferol-3-O-glucoside (Astragalin) | LOQ         | LOD        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | 0.82±0.42  | 0.29±0.09 | LOD       |
| <b>Flavonoids</b>       | Kaempferol-3-O-glucuronic acid        | LOQ         | LOQ        | 0.38±0.02  | LOQ        | 0.37±0.04  | LOQ        | 0.44±0.08  | 0.64±0.33  | LOQ       | 0.32±0.10 |
| <b>Flavonoids</b>       | Luteolin                              | LOQ         | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       | LOD       |
| <b>Flavonoids</b>       | Quercetin                             | 0.56±0.10   | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       | LOD       |
| <b>Flavonoids</b>       | Quercetin-3-O-glucoside               | 0.75±0.05   | LOQ        | 0.27±0.00  | 0.23±0.07  | 0.38±0.08  | LOQ        | 0.30±0.07  | 0.17±0.07  | 0.26±0.01 | 0.15±0.04 |
| <b>Lignans</b>          | Matairesinol                          | 0.22±0.07   | LOQ        | LOQ        | LOQ        | LOQ        | LOD        | LOQ        | LOQ        | LOD       | LOD       |
| <b>Lignans</b>          | Pinoresinol                           | LOQ         | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | 0.24±0.14  | LOD       | LOD       |
| <b>Lignans</b>          | Secoisolariciresinol                  | LOQ         | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       | LOD       |
| <b>Phenolic Acids</b>   | 1,3-Dicaffeoylquinic acid             | 0.20±0.03   | 0.30±0.04  | LOQ        | LOQ        | LOQ        | LOD        | LOD        | LOD        | LOD       | LOD       |
| <b>Phenolic Acids</b>   | 3,4-Dimethoxycinnamic acid            | 0.41±0.10   | 0.31±0.02  | 0.29±0.03  | 0.42±0.07  | 0.86±0.05  | 0.76±0.07  | 0.61±0.04  | 0.65±0.02  | 0.40±0.07 | LOD       |
| <b>Phenolic Acids</b>   | 4-hydroxybenzoic acid                 | LOQ         | LOQ        | 0.20±0.03  | 0.17±0.04  | 0.36±0.08  | 0.31±0.11  | 0.17±0.05  | 0.25±0.08  | 0.31±0.11 | 0.29±0.11 |
| <b>Phenolic Acids</b>   | 4-O-Caffeoylquinic acid               | 0.51±0.14   | 0.13±0.06  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD       | LOD       |
| <b>Phenolic Acids</b>   | Caffeic acid                          | 0.25±0.08   | 0.12±0.06  | LOQ        | LOQ        | 0.11±0.01  | LOQ        | LOD        | 0.10±0.03  | LOD       | LOD       |
| <b>Phenolic Acids</b>   | Caffeic acid hexoside                 | 4.62±2.26   | 0.85±0.21  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD       | LOD       |
| <b>Phenolic Acids</b>   | Chlorogenic acid                      | 0.54±0.09   | LOQ        | 0.76±0.19  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD       | LOD       |
| <b>Phenolic Acids</b>   | cis Cinnamic acid                     | 10.39±3.37  | 2.44±1.19  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD       | LOD       |
| <b>Phenolic Acids</b>   | Ellagic acid                          | 1.51±0.69   | 0.20±0.08  | 1.77±0.66  | 0.26±0.01  | 0.37±0.06  | LOQ        | 0.29±0.09  | 1.06±0.70  | 0.24±0.03 | 0.25±0.14 |



|                       |                               |            |            |            |            |            |            |            |            |            |           |
|-----------------------|-------------------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|-----------|
| <b>Phenolic Acids</b> | Ferulic acid                  | 2.81±0.69  | 0.90±0.31  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD        | LOD        | LOD       |
| <b>Phenolic Acids</b> | p-coumaric acid               | 0.67±0.18  | 0.04±0.01  | 1.19±0.27  | 0.83±0.10  | 0.38±0.18  | 0.56±0.12  | 0.57±0.14  | 0.33±0.27  | 0.38±0.10  | 0.33±0.20 |
| <b>Phenolic Acids</b> | Protocatechuic acid           | 23.17±1.4  | 23.73±3.7  | 24.45±1.4  | 30.93±2.7  | 15.59±0.6  | 14.14±1.2  | 15.29±1.0  | 14.69±1.0  | 11.31±1.0  | 7.48±5.38 |
| <b>Phenolic Acids</b> | Quinic acid                   | 0.20±0.03  | 0.30±0.04  | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Linseed Oil</b>    |                               |            |            |            |            |            |            |            |            |            |           |
| <b>Flavan-3-Ol</b>    | Gallic acid                   | 0.40±0.11  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD        | LOD       |
| <b>Flavones</b>       | Amentoflavone                 | LOQ        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Flavones</b>       | Herbacetin                    | 36.21±4.84 | 11.70±2.15 | 5.39±0.82  | 1.21±0.22  | 2.40±0.36  | 1.81±0.26  | 1.99±0.26  | 1.75±0.33  | 1.67±0.31  | 1.29±0.37 |
| <b>Flavonoids</b>     | Apigenin                      | LOQ        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Flavonoids</b>     | Chalcone                      | LOQ        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Flavonoids</b>     | Eriodictyol                   | 0.34±0.07  | LOQ        | 0.35±0.07  | LOQ        | 0.19±0.07  | LOQ        | LOQ        | 0.49±0.08  | LOQ        | LOQ       |
| <b>Flavonoids</b>     | Hesperetin                    | 3.22±1.22  | 1.36±0.25  | 0.33±0.08  | LOQ        | LOQ        | LOQ        | LOD        | LOQ        | LOD        | LOQ       |
| <b>Flavonoids</b>     | Isorhamnetin                  | 0.55±0.19  | 0.21±0.08  | LOQ        | 0.30±0.04  | LOQ        | 0.37±0.12  | 0.31±0.06  | LOQ        | LOQ        | LOQ       |
| <b>Flavonoids</b>     | 3-rutinoside                  | 0.52±0.07  | 0.39±0.14  | 0.46±0.17  | 0.40±0.00  | 0.35±0.05  | 0.31±0.04  | 0.34±0.02  | LOQ        | LOQ        | LOQ       |
| <b>Flavonoids</b>     | Kaempferol                    | 0.25±0.09  | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Flavonoids</b>     | Luteolin                      | 0.17±0.05  | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Flavonoids</b>     | Luteolin-7-O-glucoside        | 0.20±0.09  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD        | LOQ        | LOQ        | LOQ       |
| <b>Flavonoids</b>     | Naringin                      | 0.40±0.11  | 0.27±0.08  | LOQ        | 0.35±0.09  | LOQ        | 0.28±0.09  | 0.26±0.00  | 0.37±0.13  | LOQ        | LOQ       |
| <b>Flavonoids</b>     | Quercetin-3-O-glucuronic acid | 0.29±0.09  | 0.47±0.12  | 0.46±0.10  | LOQ        | 0.24±0.07  | 0.31±0.12  | 0.52±0.24  | 0.62±0.37  | LOQ        | LOQ       |
| <b>Lignans</b>        | Matairesinol                  | 0.28±0.07  | LOQ        | LOQ        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Lignans</b>        | Pinoresinol                   | 5.24±1.88  | 1.44±0.24  | 0.45±0.07  | 0.11±0.02  | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Lignans</b>        | Secoisolaricresinol           | LOQ        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Phenolic Acids</b> | 1,3-Dicaffeoylquinic acid     | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ       |
| <b>Phenolic Acids</b> | 3,4-Dimethoxycinnamic acid    | LOQ        | 0.22±0.02  | LOQ        | 0.34±0.08  | 0.49±0.04  | 0.53±0.08  | 0.44±0.06  | 0.34±0.03  | LOQ        | LOD       |
| <b>Phenolic Acids</b> | 4-hydroxybenzoic acid         | 0.95±0.30  | 0.35±0.05  | LOQ        | 0.31±0.07  | LOQ        | LOQ        | 0.37±0.07  | LOQ        | 0.38±0.12  | 0.41±0.06 |
| <b>Phenolic Acids</b> | Caffeic acid                  | 0.36±0.07  | LOQ        | 0.38±0.12  | 0.35±0.12  | 0.60±0.09  | 0.39±0.08  | 0.38±0.02  | 0.49±0.09  | 0.36±0.10  | LOQ       |
| <b>Phenolic Acids</b> | Caffeic acid hexoside         | 0.34±0.07  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD        | LOD       |
| <b>Phenolic Acids</b> | Chlorogenic acid              | 0.37±0.07  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD        | LOD       |
| <b>Phenolic Acids</b> | cis Cinnamic acid             | 0.71±0.24  | LOQ        | LOQ        | LOQ        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Phenolic Acids</b> | Coumaric acid hexoside        | 0.11±0.01  | 0.03±0.01  | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD        | LOD       |
| <b>Phenolic Acids</b> | Ellagic acid                  | 1.07±0.25  | LOQ        | LOQ        | 0.32±0.08  | LOQ        | LOQ        | LOQ        | LOD        | 0.32±0.01  | LOQ       |
| <b>Phenolic Acids</b> | Ferulic acid                  | 1.24±0.26  | 0.23±0.07  | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOQ        | LOD       |
| <b>Phenolic Acids</b> | p-coumaric acid               | 6.07±2.55  | 1.09±0.23  | 1.57±0.18  | 0.82±0.11  | 0.44±0.15  | 0.51±0.11  | 0.48±0.11  | 0.58±0.18  | 0.41±0.07  | LOQ       |
| <b>Phenolic Acids</b> | Quinic acid                   | 22.74±1.86 | 26.48±1.35 | 23.36±1.39 | 22.92±1.31 | 15.37±1.17 | 13.43±1.17 | 15.40±0.80 | 15.15±1.04 | 12.28±0.76 | 5.23±3.01 |

|                       |                                       |            |            |             |             |             |             |             |             |             |             |
|-----------------------|---------------------------------------|------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| <b>Hempseed Oil</b>   |                                       |            |            |             |             |             |             |             |             |             |             |
|                       |                                       |            |            | 1.78 ±      | 0.74 ±      | 1.95 ±      | 0.99 ±      | 0.71 ±      | 0.94 ±      | 1.88 ±      | 2.34 ±      |
| <b>Flavan-3-Ol</b>    | Epicatechin                           | 3.0 ± 1.2  | 1.9 ± 0.6  | 0.46        | 0.29        | 0.17        | 0.14        | 0.28        | 0.03        | 0.02        | 0.63        |
| <b>Flavan-3-Ol</b>    | Gallocatechol                         | 1.3 ± 0.3  | 0.3 ± 0.1  | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOQ         | LOD         | LOD         |
| <b>Flavones</b>       | Amentoflavone                         | 0.3 ± 0.1  | LOD        | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                       |                                       |            |            | 1.72 ±      | 0.19 ±      | 0.41 ±      |             | 0.48 ±      | 0.35 ±      | 0.37 ±      | 0.25 ±      |
| <b>Flavones</b>       | Herbacetin                            | 19.0 ± 4.3 | 13.6 ± 2.9 | 0.44        | 0.04        | 0.11        | 0.4 ± 0.06  | 0.17        | 0.01        | 0.13        | 0.07        |
| <b>Flavonoids</b>     | Apigenin                              | 0.1 ± 0.0  | 0.0 ± 0.0  | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                       |                                       |            |            | 0.14 ±      | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
| <b>Flavonoids</b>     | Chalcone                              | 0.1 ± 0.0  | 0.1 ± 0.0  | 0.03        |             |             |             |             |             |             |             |
|                       |                                       |            |            | 0.37 ±      |             |             |             |             |             |             |             |
| <b>Flavonoids</b>     | Eriodictyol                           | 0.3 ± 0.1  | LOQ        | 0.09        | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOD         |
|                       |                                       |            |            | 0.98 ±      | 0.75 ±      | 0.85 ±      |             |             |             |             |             |
| <b>Flavonoids</b>     | Hesperetin                            | 7.1 ± 1.7  | 2.2 ± 0.9  | 0.47        | 0.43        | 0.39        | LOQ         | LOQ         | LOQ         | LOQ         | LOD         |
|                       |                                       |            |            | 0.42 ±      |             |             |             |             |             |             |             |
| <b>Flavonoids</b>     | Hesperidin                            | 2.0 ± 0.8  | 0.3 ± 0.1  | 0.08        | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOQ         |
|                       |                                       |            |            |             |             |             |             | 0.48 ±      | 0.36 ±      | 0.27 ±      | 0.19 ±      |
| <b>Flavonoids</b>     | Isorhamnetin                          | 4.1 ± 0.8  | 0.5 ± 0.1  | LOQ         | LOQ         | 2.4 ± 1.25  | LOQ         | 0.22        | 0.07        | 0.09        | 0.14        |
|                       |                                       |            |            |             |             |             |             |             |             |             |             |
| <b>Flavonoids</b>     | Isorhamnetin-3-O-glucoside            | 1.0 ± 0.4  | LOQ        | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         |
|                       |                                       |            |            | 0.42 ±      | 0.59 ±      | 0.78 ±      |             |             |             |             |             |
| <b>Flavonoids</b>     | Kaempferol                            | 1.4 ± 0.2  | 0.4 ± 0.1  | 0.09        | 0.24        | 0.35        | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
|                       |                                       |            |            |             |             |             |             |             | 0.44 ±      |             |             |
| <b>Flavonoids</b>     | Kaempferol-3-O-arabinoside            | 4.9 ± 0.3  | 1.8 ± 0.5  | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | 0.04        | LOQ         | LOQ         |
|                       |                                       |            |            |             |             |             |             |             |             |             |             |
| <b>Flavonoids</b>     | Kaempferol-3-O-glucoside (Astragalin) | 0.7 ± 0.2  | 0.3 ± 0.0  | LOQ         | 0.29 ± 0.0  | 0.49 ± 0.06 | 0.42 ± 0.15 | 0.34 ± 0.07 | 0.36 ± 0.13 | LOQ         | LOQ         |
|                       |                                       |            |            |             |             |             |             |             |             |             |             |
| <b>Flavonoids</b>     | Kaempferol-3-O-glucuronic acid        | 0.5 ± 0.2  | 0.2 ± 0.0  | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOQ         | LOD         | LOQ         |
| <b>Flavonoids</b>     | Luteolin                              | LOQ        | LOD        | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                       |                                       |            |            |             |             |             |             |             |             |             |             |
| <b>Flavonoids</b>     | Luteolin-7-O-glucoside                | 0.3 ± 0.2  | LOQ        | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
| <b>Flavonoids</b>     | Naringenin                            | 0.2 ± 0.0  | LOQ        | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         |
| <b>Flavonoids</b>     | Naringin                              | 1.7 ± 0.0  | LOQ        | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         |
| <b>Flavonoids</b>     | Quercetin                             | LOQ        | LOQ        | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
|                       |                                       |            |            |             |             |             |             |             |             |             |             |
| <b>Flavonoids</b>     | Quercetin-3-O-glucuronic acid         | 0.8 ± 0.3  | 0.3 ± 0.1  | LOQ         | 0.25 ± 0.03 | 0.36 ± 0.13 | LOQ         | LOQ         | 0.41 ± 0.11 | 0.23 ± 0.08 | 0.29 ± 0.12 |
| <b>Lignans</b>        | Lariciresinol                         | 0.1 ± 0.0  | 0.0 ± 0.0  | 0.07 ± 0.0  | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                       |                                       |            |            | 1.13 ±      | 1.16 ±      | 3.11 ±      | 0.54 ±      | 2.24 ±      | 0.73 ±      |             |             |
| <b>Lignans</b>        | Matairesinol                          | 2.1 ± 0.2  | 1.3 ± 0.4  | 0.39        | 0.43        | 0.65        | 0.17        | 1.01        | 0.22        | LOQ         | LOD         |
| <b>Lignans</b>        | Pinoresinol                           | 1.6 ± 0.1  | 0.6 ± 0.2  | 0.4 ± 0.15  | 0.7 ± 0.32  | LOQ         | LOQ         | LOQ         | LOQ         | LOD         | LOD         |
|                       |                                       |            |            | 0.38 ±      |             |             |             |             |             |             |             |
| <b>Lignans</b>        | Secoisolariciresinol                  | 5.5 ± 2.6  | 1.1 ± 0.4  | 0.19        | LOQ         | LOQ         | LOQ         | LOD         | LOD         | LOQ         | LOQ         |
|                       |                                       |            |            |             |             |             |             |             |             | LOD         | LOD         |
|                       |                                       |            |            |             |             |             |             |             |             |             |             |
| <b>Phenolic Acids</b> | 3,4-Dimethoxycinnamic acid            | LOQ        | LOQ        | 0.27 ± 0.08 | 0.36 ± 0.1  | 0.82 ± 0.13 | 0.84 ± 0.05 | 0.68 ± 0.06 | 0.65 ± 0.06 |             |             |
|                       |                                       |            |            |             |             |             |             |             |             |             |             |
| <b>Phenolic Acids</b> | 4-O-Caffeoylquinic acid               | 0.2 ± 0.1  | LOD        | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                       |                                       |            |            |             |             |             |             |             |             |             |             |
| <b>Phenolic Acids</b> | 4-hydroxybenzoic acid                 | 2.0 ± 0.4  | 0.3 ± 0.1  | LOQ         | 0.98 ± 0.51 | 1.36 ± 0.86 | 0.31 ± 0.06 | LOQ         | 0.36 ± 0.05 | LOQ         | LOQ         |
| <b>Phenolic Acids</b> | Caffeic acid                          | LOQ        | LOQ        | LOQ         | LOQ         | LOD         | LOD         | LOD         | LOD         | 0.19 ± 0.1  | LOD         |
|                       |                                       |            |            | 0.48 ±      |             | 1.07 ±      |             |             | 0.38 ±      |             |             |
| <b>Phenolic Acids</b> | Caffeic acid hexoside                 | 1.6 ± 0.3  | 0.6 ± 0.2  | 0.15        | 0.6 ± 0.28  | 0.46        | LOQ         | 0.46 ± 0.2  | 0.13        | LOQ         | LOD         |
|                       |                                       |            |            | 0.46 ±      |             |             |             | 0.44 ±      |             | LOD         | LOD         |
| <b>Phenolic Acids</b> | Chlorogenic acid                      | 3.5 ± 2.4  | 0.7 ± 0.3  | 0.19        | LOQ         | LOQ         | LOQ         | 0.08        | LOQ         |             |             |
| <b>Phenolic Acids</b> | Coumaric acid hexoside                | 0.1 ± 0.0  | LOD        | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         | LOD         |
|                       |                                       |            |            |             |             |             |             |             |             |             |             |
| <b>Phenolic Acids</b> | Ellagic acid                          | 2.4 ± 1.1  | 0.7 ± 0.1  | 0.17 ± 0.02 | 0.75 ± 0.24 | 0.46 ± 0.23 | LOQ         | 0.18 ± 0.01 | 0.34 ± 0.09 | 1.52 ± 0.32 | 0.23 ± 0.05 |

|                |                   |            |            |              |              |              |             |              |              |             |             |
|----------------|-------------------|------------|------------|--------------|--------------|--------------|-------------|--------------|--------------|-------------|-------------|
| Phenolic Acids | Ferulic acid      | 1.1 ± 0.2  | LOQ        | LOQ          | LOQ          | LOQ          | LOQ         | LOD          | LOD          | LOD         | LOD         |
| Phenolic Acids | Gallic acid       | 0.6 ± 0.2  | LOQ        | LOQ          | LOQ          | LOQ          | LOQ         | LOQ          | LOQ          | 0.92 ± 0.43 | 0.82 ± 0.25 |
| Phenolic Acids | Quinic acid       | 25.3 ± 1.1 | 22.1 ± 1.5 | 20.93 ± 1.12 | 21.15 ± 5.01 | 12.51 ± 1.38 | 12.0 ± 0.83 | 12.66 ± 1.39 | 12.21 ± 1.23 | 8.91 ± 2.36 | 4.23 ± 3.08 |
| Phenolic Acids | cis Cinnamic acid | 1.3 ± 0.3  | LOQ        | LOQ          | LOQ          | LOQ          | LOD         | LOD          | LOD          | LOD         | LOD         |
| Phenolic Acids | p-coumaric acid   | 7.2 ± 2.7  | 1.8 ± 0.8  | 4.03 ± 1.7   | 2.84 ± 0.98  | 1.37 ± 0.72  | 0.8 ± 0.23  | 0.85 ± 0.21  | 1.25 ± 0.47  | 0.91 ± 0.04 | 0.39 ± 0.32 |

<LOD: below limit of detection (determined with  $S/N < 3$ ), <LOQ: below limit of quantification ( $S/N < 10$ )

**Table S62:** Quantified results of the GC-MS measurements. Data given as mean ± standard deviation (SD) in ppm, n=9.

| Oil Type               | 1-Hexanol [ppm] | Hexanal [ppm] | 2-Heptanone [ppm] | 2-Pentanone [ppm] |
|------------------------|-----------------|---------------|-------------------|-------------------|
| <b>Black cumin Oil</b> |                 |               |                   |                   |
| Day 0                  | 0.00 ± 0.00     | 0.02 ± 0.00   |                   |                   |
| 3                      | 0.01 ± 0.00     | 0.08 ± 0.01   |                   |                   |
| 7                      | 0.01 ± 0.00     | 0.12 ± 0.02   |                   |                   |
| 14                     | 0.02 ± 0.01     | 0.28 ± 0.11   |                   |                   |
| 28                     | 0.06 ± 0.01     | 0.33 ± 0.12   |                   |                   |
| 42                     | 0.06 ± 0.02     | 0.66 ± 0.11   |                   |                   |
| 56                     | 0.06 ± 0.01     | 0.63 ± 0.06   |                   |                   |
| 84                     | 0.15 ± 0.04     | 1.37 ± 0.30   |                   |                   |
| 112                    | 0.17 ± 0.03     | 1.45 ± 0.21   |                   |                   |
| 168                    | 0.06 ± 0.01     | 1.17 ± 0.14   |                   |                   |
| <b>Canola Oil</b>      |                 |               |                   |                   |
| Day 0                  | 0.00 ± 0.00     | 0.02 ± 0.01   |                   |                   |
| 3                      | 0.01 ± 0.00     | 0.06 ± 0.01   |                   |                   |
| 7                      | 0.01 ± 0.00     | 0.10 ± 0.02   |                   |                   |
| 14                     | 0.02 ± 0.01     | 0.31 ± 0.06   |                   |                   |
| 28                     | 0.06 ± 0.02     | 0.45 ± 0.11   |                   |                   |
| 42                     | 0.06 ± 0.00     | 0.65 ± 0.19   |                   |                   |
| 56                     | 0.04 ± 0.02     | 0.58 ± 0.04   |                   |                   |
| 84                     | 0.08 ± 0.00     | 0.76 ± 0.17   |                   |                   |
| 112                    | 0.10 ± 0.02     | 0.84 ± 0.25   |                   |                   |
| 168                    | 0.08 ± 0.02     | 0.96 ± 0.18   |                   |                   |
| <b>Sunflower Oil</b>   |                 |               |                   |                   |
| Day 0                  | 0.02 ± 0.01     | 0.02 ± 0.00   |                   |                   |
| 3                      | 0.01 ± 0.01     | 0.04 ± 0.01   |                   |                   |
| 7                      | 0.01 ± 0.01     | 0.06 ± 0.01   |                   |                   |
| 14                     | 0.02 ± 0.00     | 0.47 ± 0.17   |                   |                   |
| 28                     | 0.06 ± 0.03     | 0.69 ± 0.21   |                   |                   |

|                      |                 |                  |                 |                 |
|----------------------|-----------------|------------------|-----------------|-----------------|
| <b>42</b>            | $0.10 \pm 0.02$ | $0.85 \pm 0.38$  |                 |                 |
| <b>56</b>            | $0.15 \pm 0.02$ | $1.24 \pm 0.29$  |                 |                 |
| <b>84</b>            | $0.11 \pm 0.02$ | $1.61 \pm 0.37$  |                 |                 |
| <b>112</b>           | $0.22 \pm 0.05$ | $1.81 \pm 0.51$  |                 |                 |
| <b>168</b>           | $0.13 \pm 0.01$ | $2.13 \pm 0.12$  |                 |                 |
| <b>HO</b>            |                 |                  |                 |                 |
| <b>Sunflower Oil</b> |                 |                  |                 |                 |
| <b>Day 0</b>         | $0.02 \pm 0.01$ | $0.02 \pm 0.00$  |                 |                 |
| <b>3</b>             | $0.02 \pm 0.01$ | $0.04 \pm 0.01$  |                 |                 |
| <b>7</b>             | $0.01 \pm 0.00$ | $0.06 \pm 0.01$  |                 |                 |
| <b>14</b>            | $0.02 \pm 0.00$ | $0.47 \pm 0.07$  |                 |                 |
| <b>28</b>            | $0.06 \pm 0.01$ | $0.83 \pm 0.13$  |                 |                 |
| <b>42</b>            | $0.10 \pm 0.03$ | $0.97 \pm 0.26$  |                 |                 |
| <b>56</b>            | $0.15 \pm 0.03$ | $1.55 \pm 0.33$  |                 |                 |
| <b>84</b>            | $0.11 \pm 0.02$ | $3.87 \pm 0.89$  |                 |                 |
| <b>112</b>           | $0.22 \pm 0.08$ | $2.17 \pm 1.09$  |                 |                 |
| <b>168</b>           | $0.13 \pm 0.03$ | $1.28 \pm 0.58$  |                 |                 |
| <b>Linseed Oil</b>   |                 |                  |                 |                 |
| <b>Day 0</b>         | $0.04 \pm 0.02$ | $0.03 \pm 0.01$  | $0.00 \pm 0.00$ |                 |
| <b>3</b>             | $0.05 \pm 0.02$ | $0.05 \pm 0.02$  | $0.01 \pm 0.00$ |                 |
| <b>7</b>             | $0.06 \pm 0.01$ | $0.08 \pm 0.03$  | $0.02 \pm 0.01$ |                 |
| <b>14</b>            | $0.12 \pm 0.03$ | $0.71 \pm 0.27$  | $0.02 \pm 0.01$ |                 |
| <b>28</b>            | $0.30 \pm 0.09$ | $5.08 \pm 1.68$  | $0.03 \pm 0.01$ |                 |
| <b>42</b>            | $0.46 \pm 0.12$ | $18.32 \pm 6.83$ | $0.06 \pm 0.02$ |                 |
| <b>56</b>            | $0.64 \pm 0.22$ | $10.51 \pm 2.61$ | $0.04 \pm 0.02$ |                 |
| <b>84</b>            | $0.58 \pm 0.07$ | $18.50 \pm 3.08$ | $0.03 \pm 0.01$ |                 |
| <b>112</b>           | $0.60 \pm 0.27$ | $13.33 \pm 2.57$ | $0.05 \pm 0.01$ |                 |
| <b>168</b>           | $0.37 \pm 0.03$ | $5.72 \pm 1.18$  | $0.02 \pm 0.00$ |                 |
| <b>Hempseed Oil</b>  |                 |                  |                 |                 |
| <b>Day 0</b>         | $0.07 \pm 0.01$ | $0.07 \pm 0.01$  | $0.01 \pm 0.00$ | $0.01 \pm 0.00$ |
| <b>3</b>             | $0.09 \pm 0.01$ | $0.10 \pm 0.02$  | $0.02 \pm 0.00$ | $0.01 \pm 0.00$ |
| <b>7</b>             | $0.09 \pm 0.00$ | $0.12 \pm 0.03$  | $0.02 \pm 0.01$ | $0.01 \pm 0.00$ |
| <b>14</b>            | $0.18 \pm 0.03$ | $0.66 \pm 0.20$  | $0.02 \pm 0.00$ | $0.01 \pm 0.00$ |
| <b>28</b>            | $0.36 \pm 0.07$ | $6.73 \pm 1.66$  | $0.03 \pm 0.00$ | $0.02 \pm 0.00$ |
| <b>42</b>            | $0.51 \pm 0.05$ | $28.87 \pm 2.86$ | $0.07 \pm 0.01$ | $0.02 \pm 0.00$ |
| <b>56</b>            | $0.78 \pm 0.16$ | $24.38 \pm 0.54$ | $0.05 \pm 0.01$ | $0.03 \pm 0.00$ |
| <b>84</b>            | $0.81 \pm 0.20$ | $27.86 \pm 3.61$ | $0.06 \pm 0.00$ | $0.04 \pm 0.00$ |
| <b>112</b>           | $0.76 \pm 0.06$ | $29.61 \pm 7.38$ | $0.08 \pm 0.01$ | $0.05 \pm 0.01$ |
| <b>168</b>           | $0.73 \pm 0.01$ | $25.77 \pm 8.12$ | $0.11 \pm 0.01$ | $0.06 \pm 0.01$ |

**Table S73:** Results of volatiles analyzed via GC-MS. Data are given as mean  $\pm$  standard deviation (SD) in area under the curve (AuC), n=9.

| Oil Type                       | Day 0                   | Day 3                   | Day 7                | Day 14                  | Day 28              | Day 42                   | Day 56                    | Day 84                   | Day 112                | Day 168               |
|--------------------------------|-------------------------|-------------------------|----------------------|-------------------------|---------------------|--------------------------|---------------------------|--------------------------|------------------------|-----------------------|
| <b>Black cummin Oil</b>        |                         |                         |                      |                         |                     |                          |                           |                          |                        |                       |
| (E)-2-Octenal                  | 1814032 $\pm$ 238846    | 1819626 $\pm$ 351657    | 3406733 $\pm$ 34603  | 376867 $\pm$ 33912      | 308100 $\pm$ 56062  | 610851 $\pm$ 115310      | 985905 $\pm$ 843218       | 1858718 $\pm$ 140982     | 3283767 $\pm$ 194190   | 6268856 $\pm$ 249311  |
| 1-Octen-3-ol                   | 1744145 $\pm$ 542885    | 1261519 $\pm$ 318186    | 207415 $\pm$ 21670   | 21198 $\pm$ 10710       | 20002 $\pm$ 4552    | 140156 $\pm$ 59396       | 71717 $\pm$ 17997         | 174247 $\pm$ 47269       | 353721 $\pm$ 70634     | 81831 $\pm$ 16019     |
| 2-Decen-1-ol                   | 1467131 $\pm$ 205479    | 2492981 $\pm$ 127244    | 110971 $\pm$ 1521    | 71377 $\pm$ 7421        | 98568 $\pm$ 9616    | 133927 $\pm$ 18467       | 224148 $\pm$ 14972        | 51126 $\pm$ 14503        | 56216 $\pm$ 18655      | 76477 $\pm$ 5706      |
| 2-Octen-1-ol                   | 324867 $\pm$ 90511      | 233405 $\pm$ 174651     | 15085 $\pm$ 5162     | 10438 $\pm$ 3011        | 19677 $\pm$ 2017    | 33918 $\pm$ 6143         | 39828 $\pm$ 8209          | 83266 $\pm$ 2794         | 103792 $\pm$ 13487     | 103937 $\pm$ 26483    |
| 6-Oxabicyclo [3.1.0]hexan-3-ol | 176555 $\pm$ 12098      | 150664 $\pm$ 7361       | 90996 $\pm$ 21127    | 137189 $\pm$ 11021      | 168276 $\pm$ 44423  | 151689 $\pm$ 31013       | 188640 $\pm$ 25270        | 197573 $\pm$ 43705       | 236859 $\pm$ 4290      | 868122 $\pm$ 396428   |
| (E)-2-Decenal                  | 12698 $\pm$ 3669        | 13464 $\pm$ 4345        | 1914710 $\pm$ 414185 | 173154 $\pm$ 15780      | 145823 $\pm$ 25496  | 3398167 $\pm$ 530742     | 6554391 $\pm$ 404072      | 800696 $\pm$ 295421      | 1520851 $\pm$ 255892   | 869496 $\pm$ 152583   |
| (E)-2-Nonenal                  | 134810 $\pm$ 86201      | 185971 $\pm$ 54526      | 945660 $\pm$ 111820  | 1314165 $\pm$ 147815    | 1163775 $\pm$ 52574 | 5487132 $\pm$ 817286     | 6956830 $\pm$ 865767      | 5146693 $\pm$ 1047062    | 4321810 $\pm$ 949947   | 2539703 $\pm$ 518056  |
| 4,5-Epoxy-(E)-2-decenal        | 319978 $\pm$ 25265      | 168363 $\pm$ 17967      | 189464 $\pm$ 35918   | 188188 $\pm$ 138084     | 132703 $\pm$ 21500  | 283722 $\pm$ 89344       | 440474 $\pm$ 11713        | 734743 $\pm$ 127673      | 940997 $\pm$ 42384     | 2007405 $\pm$ 907617  |
| Heptanal                       | 79854 $\pm$ 20965       | 81923 $\pm$ 23740       | 1366942 $\pm$ 90819  | 2018176 $\pm$ 354175    | 715476 $\pm$ 492624 | 4339881 $\pm$ 814078     | 4165367 $\pm$ 451962      | 4960221 $\pm$ 1514536    | 7584147 $\pm$ 1760053  | 6161253 $\pm$ 346332  |
| Nonanal                        | 822840 $\pm$ 28635      | 1233942 $\pm$ 68030     | 1778205 $\pm$ 341746 | 71781448 $\pm$ 39776234 | 274953 $\pm$ 102615 | 106930668 $\pm$ 50760127 | 195928107 $\pm$ 140972936 | 494157945 $\pm$ 84525833 | 12474410 $\pm$ 1146555 | 1877601 $\pm$ 1346242 |
| 5-Ethyl-2(5)-furanone          | 43877740 $\pm$ 19955546 | 25886921 $\pm$ 10879349 | 46825 $\pm$ 13750    | 153904 $\pm$ 63349      | 72914 $\pm$ 19212   | 705269 $\pm$ 182465      | 508809 $\pm$ 46103        | 679853 $\pm$ 12182       | 1069533 $\pm$ 552839   | 513528 $\pm$ 81374    |
| <b>Canola Oil</b>              |                         |                         |                      |                         |                     |                          |                           |                          |                        |                       |
| (E)-2-Decenal                  | 1080101 $\pm$ 96764     | 1139515 $\pm$ 207639    | 1039928 $\pm$ 347859 | 113827 $\pm$ 35086      | 158089 $\pm$ 58538  | 2463322 $\pm$ 349052     | 3009709 $\pm$ 429363      | 742837 $\pm$ 2553        | 809419 $\pm$ 376572    | 476198 $\pm$ 106725   |
| (E)-2-Nonenal                  | 444296 $\pm$ 77083      | 519612 $\pm$ 91645      | 580579 $\pm$ 96322   | 1200185 $\pm$ 259314    | 872553 $\pm$ 145985 | 2530206 $\pm$ 250338     | 1463128 $\pm$ 362301      | 2244442 $\pm$ 478786     | 2078247 $\pm$ 323404   | 1464372 $\pm$ 103559  |
| 2,4-Decadienal                 | 54698 $\pm$ 11141       | 84045 $\pm$ 10177       | 143056 $\pm$ 10240   | 76369 $\pm$ 13985       | 142923 $\pm$ 5714   | 233211 $\pm$ 58694       | 256621 $\pm$ 62592        | 618702 $\pm$ 183001      | 773629 $\pm$ 4537      | 772176 $\pm$ 23228    |
| 2,4-Heptadienal                | 58649 $\pm$ 11155       | 136129 $\pm$ 9504       | 188185 $\pm$ 7929    | 208381 $\pm$ 23751      | 294508 $\pm$ 56076  | 328809 $\pm$ 41857       | 170354 $\pm$ 28311        | 304372 $\pm$ 75256       | 100409 $\pm$ 13480     | 64129 $\pm$ 9273      |
| Heptanal                       | 1174405 $\pm$ 217990    | 918220 $\pm$ 224253     | 946195 $\pm$ 286515  | 900617 $\pm$ 213011     | 880076 $\pm$ 199901 | 1505704 $\pm$ 320353     | 939369 $\pm$ 305482       | 1757077 $\pm$ 430067     | 4163854 $\pm$ 1106735  | 3298991 $\pm$ 977316  |
| <b>Sunflower Oil</b>           |                         |                         |                      |                         |                     |                          |                           |                          |                        |                       |
| Heptanal                       | 1080101 $\pm$ 326490    | 1070614 $\pm$ 329099    | 946195 $\pm$ 355624  | 900617 $\pm$ 171960     | 880076 $\pm$ 168038 | 1505704 $\pm$ 489519     | 939369 $\pm$ 270417       | 1757077 $\pm$ 338874     | 4163854 $\pm$ 1747447  | 3298991 $\pm$ 1543662 |
| (E)-2-Decenal                  | 444296 $\pm$ 201411     | 547796 $\pm$ 208946     | 1039928 $\pm$ 339028 | 113827 $\pm$ 19458      | 158089 $\pm$ 27025  | 2463322 $\pm$ 499575     | 3009709 $\pm$ 259663      | 742837 $\pm$ 257779      | 809419 $\pm$ 127689    | 476198 $\pm$ 36950    |
| (E)-2-Nonenal                  | 54698 $\pm$ 29378       | 102931 $\pm$ 22965      | 580579 $\pm$ 286210  | 1200185 $\pm$ 246690    | 872553 $\pm$ 179348 | 2530206 $\pm$ 886492     | 1463128 $\pm$ 614595      | 2244442 $\pm$ 335940     | 2078247 $\pm$ 893332   | 1464372 $\pm$ 148958  |
| 2,4-Decadienal                 | 58649 $\pm$ 36185       | 131809 $\pm$ 34984      | 143056 $\pm$ 14742   | 76369 $\pm$ 14015       | 142923 $\pm$ 26229  | 233211 $\pm$ 54175       | 256621 $\pm$ 37289        | 618702 $\pm$ 35328       | 773629 $\pm$ 147835    | 772176 $\pm$ 114263   |
| 2,4-Heptadienal                | 1174405 $\pm$ 497757    | 1124978 $\pm$ 479173    | 188185 $\pm$ 26510   | 208381 $\pm$ 65830      | 294508 $\pm$ 93038  | 328809 $\pm$ 70241       | 170354 $\pm$ 10028        | 304372 $\pm$ 102852      | 100409 $\pm$ 26009     | 64129 $\pm$ 4128      |



|                         |                    |                    |                  |                     |                      |                      |                      |                      |                      |                    |
|-------------------------|--------------------|--------------------|------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|--------------------|
| <b>HO Sunflower Oil</b> |                    |                    |                  |                     |                      |                      |                      |                      |                      |                    |
| (E)-2-Decenal           | 984690 ± 236674    | 1194980 ± 246860   | 1097157 ± 260025 | 136694 ± 14305      | 282243 ± 114608      | 3753913 ± 749236     | 4029623 ± 758437     | 864155 ± 277365      | 1004374 ± 326433     | 577736 ± 162073    |
| (E)-2-Octenal           | 194032 ± 30670     | 228689 ± 35261     | 306929 ± 42714   | 46180 ± 14474       | 163454 ± 44685       | 250053 ± 48113       | 310181 ± 98274       | 252199 ± 4030        | 327286 ± 44112       | 207102 ± 34775     |
| Decanal                 | 21774 ± 618        | 20534 ± 3858       | 25215 ± 6246     | 27408 ± 9746        | 58171 ± 12996        | 98474 ± 30375        | 168206 ± 15173       | 184658 ± 6896        | 141952 ± 26101       | 127798 ± 20971     |
| <b>Linseed Oil</b>      |                    |                    |                  |                     |                      |                      |                      |                      |                      |                    |
| Nonanal                 | 33516467 ± 2210001 | 20091557 ± 1194482 | 1980983 ± 143659 | 91444984 ± 5021449  | 55036585 ± 6233288   | 198352014 ± 34889347 | 127927303 ± 46111942 | 212563941 ± 57604708 | 38844367 ± 13791054  | 1166752 ± 231386   |
| <b>Nonanoic acid</b>    |                    |                    |                  |                     |                      |                      |                      |                      |                      |                    |
| 1-Octen-3-ol            | 99774 ± 14474      | 87449 ± 15666      | 53914 ± 17960    | 6928 ± 4641         | 18380 ± 8046         | 96436 ± 39258        | 64525 ± 11102        | 35203 ± 4371         | 81182 ± 9584         | 40426 ± 1254       |
| 2-Octen-1-ol            | 133082 ± 17322     | 133453 ± 18208     | 130138 ± 17930   | 53714 ± 12078       | 89476 ± 12244        | 149369 ± 35314       | 290474 ± 32510       | 781601 ± 26922       | 971075 ± 170854      | 970923 ± 32799     |
| 2,4-Decadienal          | 146866 ± 5929      | 159203 ± 32134     | 154656 ± 67436   | 141493 ± 31595      | 1412236 ± 271360     | 1074984 ± 244886     | 342412 ± 91487       | 478300 ± 52793       | 224861 ± 66097       | 256518 ± 3265      |
| 2,4-Heptadienal         | 10268 ± 144562     | 13589 ± 84591      | 14712 ± 18900    | 13572 ± 75255       | 21594 ± 68277        | 21362 ± 582421       | 26474 ± 158296       | 59432 ± 116072       | 62852 ± 191526       | 64040 ± 101324     |
| Heptanal                | 21778 ± 961528     | 14606 ± 935317     | 5938 ± 1147416   | 21836 ± 611530      | 27648 ± 565895       | 187700 ± 1452434     | 38491 ± 1406460      | 14259 ± 1426213      | 53826 ± 4249249      | 11529 ± 415021     |
| 2-Pentyl-furan          | 371743 ± 17422     | 540021 ± 21618     | 643333 ± 28427   | 239966 ± 68745      | 116835 ± 184641      | 624628 ± 247764      | 637511 ± 240170      | 445918 ± 242470      | 1195684 ± 130693     | 20078 ± 156757     |
| 5-Ethyl-2(5)-furanone   | 4251 ± 1689324     | 5302 ± 1752438     | 6737 ± 2275468   | 4606 ± 1708367      | 51884 ± 1407020      | 51783 ± 6542291      | 35545 ± 19729256     | 56011 ± 36163819     | 27576 ± 50346973     | 49402 ± 39397082   |
| <b>Hemseed Oil</b>      |                    |                    |                  |                     |                      |                      |                      |                      |                      |                    |
| Nonanoic acid           | 1223218 ± 397619   | 1096914 ± 333127   | 817554 ± 201530  | 127040 ± 27794      | 136284 ± 36238       | 2149909 ± 230040     | 3848733 ± 592128     | 933212 ± 72923       | 1020706 ± 378182     | 642643 ± 55333     |
| 1-Octen-3-ol            | 314657 ± 70980     | 395655 ± 69797     | 507056 ± 58995   | 754834 ± 306365     | 1111533 ± 77373      | 2728044 ± 141858     | 1859121 ± 259785     | 2631234 ± 334258     | 1968038 ± 926422     | 2185631 ± 67820    |
| (E)-2-Decenal           | 371708 ± 80150     | 426626 ± 61046     | 448727 ± 49458   | 70076 ± 8204        | 193896 ± 151669      | 488384 ± 48838       | 709795 ± 57153       | 356213 ± 39430       | 681846 ± 19126       | 1101604 ± 37214    |
| (E)-2-Heptenal          | 9183307 ± 1402293  | 5474476 ± 871949   | 1943676 ± 431459 | 53952709 ± 15757553 | 195677798 ± 43002544 | 436919646 ± 65101027 | 281337879 ± 5309877  | 400937516 ± 43054078 | 407503742 ± 53609309 | 38834800 ± 494247  |
| (E)-2-Nonenal           | 150262 ± 72968     | 93692 ± 44320      | 53860 ± 16387    | 6114 ± 1597         | 14622 ± 5013         | 91697 ± 9628         | 51744 ± 16181        | 41077 ± 2120         | 65154 ± 7202         | 46628 ± 1826       |
| (E)-2-Octenal           | 170400 ± 30246     | 154604 ± 41157     | 189982 ± 43578   | 121801 ± 36965      | 238603 ± 443         | 548294 ± 59764       | 1092007 ± 260655     | 1566335 ± 506221     | 1794963 ± 734016     | 3258130 ± 926954   |
| 2,4-Decadienal          | 484706 ± 110807    | 399039 ± 107078    | 419121 ± 118975  | 857535 ± 41610      | 1402420 ± 455836     | 1797753 ± 348764     | 1703540 ± 254131     | 1672378 ± 637558     | 1414218 ± 212876     | 1886161 ± 214613   |
| 2,4-Heptadienal         | 894444 ± 199295    | 826920 ± 147307    | 651201 ± 67323   | 926561 ± 160663     | 918660 ± 66048       | 1584736 ± 337549     | 1037978 ± 161514     | 2055061 ± 253698     | 3415795 ± 90325      | 4234906 ± 871328   |
| Heptanal                | 1535749 ± 331038   | 1668719 ± 363260   | 1791707 ± 355006 | 1842899 ± 390552    | 1433366 ± 333272     | 4607247 ± 552451     | 10238327 ± 1462851   | 14333658 ± 2202213   | 17917072 ± 3475795   | 20476654 ± 2195046 |
| 2-Pentyl-furan          | 285695 ± 70022     | 164655 ± 36489     | 36841 ± 7696     | 282912 ± 64017      | 159900 ± 34937       | 1946269 ± 275559     | 1615270 ± 229313     | 2368826 ± 395471     | 2151979 ± 414126     | 2302817 ± 213080   |
| 5-Ethyl-2(5)-furanone   | 20164 ± 4214       | 27614 ± 4064       | 29367 ± 5292     | 87909 ± 14309       | 83738 ± 22107        | 121564 ± 18127       | 187780 ± 32032       | 157448 ± 21776       | 188319 ± 35786       | 244933 ± 39209     |



## 6 CONCLUSION AND OUTLOOK

This thesis addressed challenges faced in the production and preservation of cold-pressed vegetable oils nowadays. Due to their limited oxidative stability and short shelf-life strategies across the entire value chain, from natural process-level improvements to packaging optimization and predictive quality modeling were explored. The work demonstrated that oxidative deterioration can be effectively mitigated by combining biological and sustainable, technological and analytical innovations. The five studies presented across Parts I–III collectively provide a comprehensive understanding of how oil processing and storage conditions determine nutritional quality and shelf-life.

Part I focused on natural and sustainable approaches to enhance the antioxidant potential and oxidative stability of oils through germination and green extraction. Both methods targeted the composition of oils, leveraging biological and process-level modifications rather than additive-based fortification. These studies provided the first comprehensive LC-MS/MS-based characterization of specific polyphenolic substances in oils derived from germinated seeds and in cloudy linseed oil. Moreover, the concept of colloidal stabilization through retained bioactive particles in linseed oil was demonstrated for the first time.

The germination study revealed that controlled sprouting of linseeds and sunflower seeds significantly increased antioxidant contents, particularly polyphenolic compounds, tocopherols, carotenoids and chlorophyll. LC-MS/MS analyses showed that up to 69 phenolic substances could be identified in germinated seed oils and antioxidant capacities increased several-fold compared to conventional oils. Rancimat measurements confirmed that the predicted shelf-life of germinated linseed oil was more than four times longer. These results highlighted germination as a viable, low-cost, and natural pre-processing step to enhance nutritional properties. However, before oils from germinated seeds can be considered safe for commercial or dietary application, potential anti-nutritional factors such as cyanogenic glycosides, protease inhibitors or residual enzymatic activities must be systematically analyzed and minimized. A comprehensive safety and compositional assessment is therefore essential to ensure that no unintended adverse effects are introduced as well.

Complementing this, the cloudy linseed oil study dealt with a green extraction process that retained bioactive-rich press cake particles in the oil, creating a novel “cloudy” linseed oil similar to non-filtered extra virgin olive oil. The cloudy oil after green extraction showed a 6.3-fold increase in total polyphenols and a 52% improvement in oxidative induction time compared to conventional linseed oil. Moreover, volatile profiling by SPME-GC-MS demonstrated a 93% reduction in hexanal and increased formation of flavor-relevant alcohols, which could also influence the flavor and may mask the typical bitter taste for linseed oil. These findings underscored the synergistic role of suspended particles in stabilizing the oil through antioxidant mechanisms. The approach also valorized linseed press cake, a typical by-product of oil pressing, which is mostly discarded to waste.

Together, the two studies showed that seed germination and by-product valorization can markedly improve the nutritional quality and oxidative stability of cold-pressed oils without chemical additives or refining. These approaches could contribute to the development of functional, clean-label oils with higher consumer acceptance and environmental sustainability.

Part II explored more midstream interventions aiming at preserving oil quality during storage by altering the oil composition through natural phospholipid fortification and by optimizing the packaging environment by using bioplastic alternatives.

The packaging material study complemented this part by evaluating how different plastics influence lipid oxidation kinetics and migration phenomena in sunflower oil. Conventional (PET, r-PET, PE, bio-PE) and biodegradable PLA bottles were compared under 56 days of simulated retail conditions. Peroxide and NMR analyses demonstrated that oils stored in PLA bottles exhibited oxidative stability comparable to PET up to two months, outperforming PE and bio-PE, which showed rapid peroxide formation due to higher oxygen permeability. Moreover, no non-intentionally added substances (NIAS) were detected in PLA, while benzene migrated from PET and r-PET at trace levels ( $\sim 0.15 \mu\text{g/g}$ ). This work provided the first comprehensive evidence that PLA represents a safe, renewable and functionally equivalent alternative to PET for edible oil packaging.

The aminophospholipid fortification study demonstrated for the first time that 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (PE(16:0/18:1)) acts as a temperature-dependent antioxidant in cold-pressed oils. Fortification with  $400 \mu\text{g/g}$  PE increased the OIT

under accelerated oxidation conditions by up to 50%, evidencing its efficacy at elevated temperatures. Under household storage, PE fortification led to lower LOOH accumulation and a significant reduction in aldehydes, particularly (E,E)-2,4-alkadienals.  $^1\text{H}$  NMR and GC-MS analyses indicated that PE(16:0/18:1) might contribute to LOOH decomposition and carbonyl scavenging, mechanisms previously proposed for aminophospholipids, while partially preserving  $\gamma$ -tocopherol levels in flaxseed oil. These results suggest a temperature-dependent antioxidant behavior, dependent on the oxidative environment and may position aminophospholipids as promising bio-inspired stabilizers for PUFA-rich oils.

In combination, these studies emphasize that midstream quality preservation relies on both oil–additive and oil–material interactions. Natural phospholipid fortification and biodegradable packaging converged as strategies that enhanced oxidative stability, could reduce waste and promote sustainable product design. Their combined application could open doors for the development of eco-functional packaging systems.

Part III advanced from stabilization strategies toward predictive understanding, aiming to quantify and determine degradation markers in cold-pressed oils. A six-month storage study of six cold-pressed oil types under household-relevant conditions provided an extensive dataset encompassing fatty acid profiles (GC-FID), polyphenol decrease (LC-MS/MS) and secondary lipid oxidation products (SPME-GC-MS). This dataset was analyzed using a systematic ML–based biomarker identification framework integrating LASSO and RFR.

The results revealed that oxidative deterioration is oil-specific, driven by the interplay of fatty acid composition, antioxidant capacity and other matrix effects. Across all oils, linoleic and  $\alpha$ -linolenic acid degradation emerged as the most consistent predictors of stability, while the accumulation of hexanal, 2,4-deca-dienal and (E)-2-nonenal represented key volatile markers of advanced oxidation. Although polyphenols were generally ranked lower, several oil-type-specific compounds exhibited strong potential as degradation biomarkers as e.g., caffeic acid in HO sunflower oil, p-coumaric acid in linseed oil, and chlorogenic acid in hempseed oil. The scoring system derived from statistical tools enabled the exploration of oil-type-specific deterioration fingerprints, offering a better prediction framework than single-marker approaches.

By demonstrating that oil stability can be modeled through multi-class, time-resolved biomarkers, this part may lay the foundation for predictive analytics in food lipidomics. Such models could be further integrated in rapid, non-invasive scanning technologies as e.g., e-nose, NIR or fluorescence-based monitoring. Furthermore, this approach bridges analytical chemistry and data science, which might enable real-time shelf-life prediction and more sustainable quality control systems in the oil industry in the near future.

To conclude, the three parts of this thesis established integrated approaches for improving, prolonging and predicting the quality of edible oils. The thesis demonstrates that natural bioprocessings (germination, green extraction), bio-based stabilization (aminophospholipids, biodegradable packaging material) and analytical modeling (biomarker identification) can be combined to form new strategies for the entire oil value chain. Future work should aim to translate these findings into industrial applications and *in-situ* sensing tools. The integration of real-time analytical techniques such as NIR spectroscopy or e- nose with predictive biomarker models could enable rapid classification of oil freshness and authenticity. Additionally, the exploration of synergistic effects between polyphenols, phospholipids and packaging materials could generate new functional packaging solutions with embedded antioxidant or oxygen-scavenging capacities.

## References

- Akoh, C. C.; Min, D. B. (2002): Food Lipids: CRC Press. DOI: 10.1201/9780203908815.
- Aktar, T., Adal, E. (2019): Determining the Arrhenius Kinetics of Avocado Oil: Oxidative Stability under Rancimat Test Conditions. In: *Foods (Basel, Switzerland)* 8 (7). DOI: 10.3390/foods8070236.
- AOCS (1995): American Oil Chemists' Society, Official methods and recommended practices. DOI: 10.1002/lipi.19970990510.
- AOCS (2017): Official method Cd 3d-63: Acid value of animal and vegetable fats and oils. Official Methods And Recommended Practices of the American Oil Chemists' Society., Urbana, IL, USA.
- Arthur, C. L.; Pawliszyn, J. (1990): Solid phase microextraction with thermal desorption using fused silica optical fibers. In: *Anal. Chem.* 62 (19), pp. 2145–2148. DOI: 10.1021/ac00218a019.
- Aschemann-Witzel, J.; Asioli, D.; Banovic, M.; Perito, M. A.; Peschel, A. O.; Stancu, V. (2023): Defining upcycled food: The dual role of upcycling in reducing food loss and waste. In: *Trends in Food Science & Technology* 132, pp. 132–137. DOI: 10.1016/j.tifs.2023.01.001.
- Belitz, HD., Grosch, W., Schieberle, P. (2008): Food Chemistry. 6th, completely revised ed. Berlin, Heidelberg: Springer Berlin Heidelberg (SpringerLink Books). DOI: 10.1007/978-3-540-73202-0.
- Benzie, I. F.; Strain, J. J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. In: *Analytical biochemistry* 239 (1), pp. 70–76. DOI: 10.1006/abio.1996.0292.
- Brand-Williams, W.; Cuvelier, M. E.; Berset, C. (1995): Use of a free radical method to evaluate antioxidant activity. In: *LWT - Food Science and Technology* 28 (1), pp. 25–30. DOI: 10.1016/S0023-6438(95)80008-5.
- Breiman, L. (2001): Random Forests. In: *Machine Learning* 45 (1), pp. 5–32. DOI: 10.1023/A:1010933404324.
- Chaiyasit, W., Elias, R. J., McClements, D. J., Decker, E. A. (2007): Role of physical structures in bulk oils on lipid oxidation. In: *Critical reviews in food science and nutrition* 47 (3), pp. 299–317. DOI: 10.1080/10408390600754248.

Choe, E., Min, D. B. (2006): Mechanisms and Factors for Edible Oil Oxidation. In: *Comp Rev Food Sci Food Safe* 5 (4), pp. 169–186. DOI: 10.1111/j.1541-4337.2006.00009.x.

Codex standard for named vegetable oils (1999). (CODEX-STAN 210-1999). FAO/WHO, Rome. Available at: <https://www.fao.org/fao-who-codexalimentarius>

Cook, R. D., Forzani, L. (2018): Big data and partial least-squares prediction. In: *Can J Statistics* 46 (1), pp. 62–78. DOI: 10.1002/cjs.11316.

Crippa, M., Solazzo, E.; Guizzardi, D., Monforti-Ferrario, F., Tubiello, F. N., Leip, A. (2021): Food systems are responsible for a third of global anthropogenic GHG emissions. In: *Nature food* 2 (3), pp. 198–209. DOI: 10.1038/s43016-021-00225-9.

Depenbusch, L., Klasen, S. (2019): The effect of bigger human bodies on the future global calorie requirements. In: *PLoS one* 14 (12), e0223188. DOI: 10.1371/journal.pone.0223188.

Di Lorenzo, C., Colombo, F., Biella, S., Stockley, C., Restani, P. (2021): Polyphenols and Human Health: The Role of Bioavailability. In: *Nutrients* 13 (1). DOI: 10.3390/nu13010273.

Esteki, M., Shahsavari, Z., Simal-Gandara, J. (2020): Gas Chromatographic Fingerprinting Coupled to Chemometrics for Food Authentication. In: *Food Reviews International* 36 (4), pp. 384–427. DOI: 10.1080/87559129.2019.1649691.

Eurostat (2024): Food waste and food waste prevention by NACE Rev. 2 activity – tonnes of fresh mass. Available at: <https://ec.europa.eu/eurostat>

Food and Agriculture Organization of the United Nations (2013): The multiple dimensions of food security. In *The state of food insecurity in the world 2013*. Rome: FAO.

Farmer, E. H., Bloomfield, G. F., Sundralingam, A., Sutton, D. A. (1942): The course and mechanism of autoxidation reactions in olefinic and polyolefinic substances, including rubber. In: *Trans. Faraday Soc.* 38, p. 348. DOI: 10.1039/TF9423800348.

Foote, C. S. (1968): Mechanisms of photosensitized oxidation. There are several different types of photosensitized oxidation which may be important in biological systems. In: *Science (New York, N.Y.)* 162 (3857), pp. 963–970. DOI: 10.1126/science.162.3857.963.

Frankel, E. N. (1980): Lipid oxidation. In: *Progress in lipid research* 19 (1-2), pp. 1–22. DOI: 10.1016/0163-7827(80)90006-5.

Frankel, E. N. (2005): Lipid oxidation. 2. ed. Bridgwater: Oily Press (Oily Press lipid library, 18).



Fruehwirth, S., Zehentner, S., Salim, M., Sterneder, S., Tiroch, J., Lieder, B. et al. (2020): In Vitro Digestion of Grape Seed Oil Inhibits Phospholipid-Regulating Effects of Oxidized Lipids. In: *Biomolecules* 10 (5). DOI: 10.3390/biom10050708.

Gliszczynska-Swiglo, A., Sikorska, E., Khmelinskii, I., Sikorski, I. (2007): Tocopherol content in edible plant oils. *Polish Journal Of Food And Nutrition Sciences*, 57 (2007), pp. 157-161.

Organisation for Economic Co-operation and Development. (2022). Global plastics outlook: Economic drivers, environmental impacts and policy options. Paris: OECD. DOI: 10.1787/de747aef-en.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F. et al. (2010): Food security: the challenge of feeding 9 billion people. In: *Science (New York, N.Y.)* 327 (5967), pp. 812–818. DOI: 10.1126/science.1185383.

Gonzalez Viejo, C., Fuentes, S. (2022): Digital Detection of Olive Oil Rancidity Levels and Aroma Profiles Using Near-Infrared Spectroscopy, a Low-Cost Electronic Nose and Machine Learning Modelling. In: *Chemosensors* 10 (5), p. 159. DOI: 10.3390/chemosensors10050159.

Grajzer, M., Szmalczel, K., Kuźmiński, Ł., Witkowski, M., Kulma, A., Prescha, A. (2020): Characteristics and Antioxidant Potential of Cold-Pressed Oils-Possible Strategies to Improve Oil Stability. In: *Foods (Basel, Switzerland)* 9 (11). DOI: 10.3390/foods9111630.

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. In: *Journal of agricultural and food chemistry* 67 (36), pp. 10174–10184. DOI: 10.1021/acs.jafc.9b03601.

Habib, M., Singh, S., Jan, S., Jan, K., Bashir, K. (2025): The future of the future foods: understandings from the past towards SDG-2. In: *NPJ science of food* 9 (1), p. 138. DOI: 10.1038/s41538-025-00484-x.

Hassoun, A., Aït-Kaddour, A., Abu-Mahfouz, A. M., Rathod, N. B., Bader, F., Barba, F. J. et al. (2023): The fourth industrial revolution in the food industry-Part I: Industry 4.0 technologies. In: *Critical reviews in food science and nutrition* 63 (23), pp. 6547–6563. DOI: 10.1080/10408398.2022.2034735.

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. In: *Grasas aceites* 66 (1), e057. DOI: 10.3989/gya.0463141.

- Kabutey, A., Mizera, Č., Herák, D. (2024): Evaluation of percentage oil yield, energy requirement and mechanical properties of selected bulk oilseeds under compression loading. In: *Journal of Food Engineering* 360, p. 111719. DOI: 10.1016/j.jfoodeng.2023.111719.
- Kamilaris, A., Fonts, A., Prenafeta-Boldú, F. X. (2019): The rise of blockchain technology in agriculture and food supply chains. In: *Trends in Food Science & Technology* 91, pp. 640–652. DOI: 10.1016/j.tifs.2019.07.034.
- Buchholz K. (2025): No End to World Hunger by 2030. Statista. Available at: <https://www.statista.com/chart/15477/the-number-of-malnourished-people-worldwide/>.
- Kharbach, M., Alaoui Mansouri, M., Taabouz, M., Yu, H. (2023): Current Application of Advancing Spectroscopy Techniques in Food Analysis: Data Handling with Chemometric Approaches. In: *Foods (Basel, Switzerland)* 12 (14). DOI: 10.3390/foods12142753.
- Lall, R. K., Proctor, A., Jain, V. P. (2009): A Rapid, Micro FAME Preparation Method for Vegetable Oil Fatty Acid Analysis by Gas Chromatography. In: *J Americ Oil Chem Soc* 86 (4), pp. 309–314. DOI: 10.1007/s11746-009-1359-6.
- Lampi, AM, Yang, Z., Mustonen, O., Piironen, V. (2020): Potential of faba bean lipase and lipoxygenase to promote formation of volatile lipid oxidation products in food models. In: *Food Chemistry* 311, p. 125982. DOI: 10.1016/j.foodchem.2019.125982.
- Li, D., Bai, L., Wang, R., Ying, S. (2024a): Research Progress of Machine Learning in Extending and Regulating the Shelf Life of Fruits and Vegetables. In: *Foods (Basel, Switzerland)* 13 (19). DOI: 10.3390/foods13193025.
- Li, Y., He, P., Shan, Y., Li, Y., Hang, Y., Shao, S. et al. (2024b): Reducing climate change impacts from the global food system through diet shifts. In: *Nat. Clim. Chang.* 14 (9), pp. 943–953. DOI: 10.1038/s41558-024-02084-1.
- Lim, H., Lee, S. Y., Kim, J. Y., Shin, Y. J., Jang, Y., Kim, H. et al. (2025): Comparison of machine learning models for classifying edible oils using Fourier-transform infrared spectroscopy. In: *Bulletin Korean Chem Soc* 46 (2), pp. 131–137. DOI: 10.1002/bkcs.12932.
- Lin, Y., Ma, J., Wang, Q., Sun, DW. (2023): Applications of machine learning techniques for enhancing nondestructive food quality and safety detection. In: *Critical reviews in food science and nutrition* 63 (12), pp. 1649–1669. DOI: 10.1080/10408398.2022.2131725.
- Loukili, N., Rosenblatt-Velin, N., Rolli, J., Levrand, S., Feihl, F., Waeber, B. et al. (2010): Oxidants positively or negatively regulate nuclear factor kappaB in a context-dependent manner. In: *The Journal of biological chemistry* 285 (21), pp. 15746–15752. DOI: 10.1074/jbc.M110.103259.

Maitin-Shepard, M. (Ed.) (2020): Innovations in the food system: Exploring the future of food – Proceedings of a workshop. Washington, DC: The National Academies Press. DOI: 10.17226/25523.

Malafeev, K. V., Apicella, A., Incarnato, L., Scarfato, P. (2023): Understanding the Impact of Biodegradable Microplastics on Living Organisms Entering the Food Chain: A Review. In: *Polymers* 15 (18). DOI: 10.3390/polym15183680.

Man, C. M. D. (2015): Shelf life. 2. ed. Chichester: Wiley-Blackwell. ISBN: 9781118694346

Mannucci, P. M., Jolliet, O., Meijaard, E., Slavin, J., Rasetti, M., Aleta, A. et al. (2023): Sustainable nutrition and the case of vegetable oils to match present and future dietary needs. In: *Frontiers in public health* 11, p. 1106083. DOI: 10.3389/fpubh.2023.1106083.

Martínez-Yusta, A., Guillén, M. D. (2014): A study by <sup>1</sup>H nuclear magnetic resonance of the influence on the frying medium composition of some soybean oil-food combinations in deep-frying. In: *Food Research International* 55, pp. 347–355. DOI: 10.1016/j.foodres.2013.11.022.

Metcalf, L. D., Schmitz, A. A. (1961): The Rapid Preparation of Fatty Acid Esters for Gas Chromatographic Analysis. In: *Anal. Chem.* 33 (3), pp. 363–364. DOI: 10.1021/ac60171a016.

Musakhanian, J., Rodier, JD, Dave, M. (2022): Oxidative Stability in Lipid Formulations: a Review of the Mechanisms, Drivers, and Inhibitors of Oxidation. In: *AAPS PharmSciTech* 23 (5), p. 151. DOI: 10.1208/s12249-022-02282-0.

Nieva-Echevarría, B., Goicoechea, E., Manzanos, M. J., Guillén, M. D. (2017): <sup>1</sup>H NMR and SPME-GC/MS study of hydrolysis, oxidation and other reactions occurring during in vitro digestion of non-oxidized and oxidized sunflower oil. Formation of hydroxy-octadecadienoates. In: *Food research international (Ottawa, Ont.)* 91, pp. 171–182. DOI: 10.1016/j.foodres.2016.11.027.

Onyeaka, H., Jalata, D. D., Mekonnen, S. A. (2023): Mitigating physical hazards in food processing: Risk assessment and preventive strategies. In: *Food science & nutrition* 11 (12), pp. 7515–7522. DOI: 10.1002/fsn3.3727.

Pérez-Gálvez, A., Viera, I., Roca, M. (2020): Carotenoids and Chlorophylls as Antioxidants. In: *Antioxidants (Basel, Switzerland)* 9 (6). DOI: 10.3390/antiox9060505.

Pignitter, M., Somoza, V. (2012): Critical evaluation of methods for the measurement of oxidative rancidity in vegetable oils. In: *Journal of Food and Drug Analysis* 20 (4). DOI: 10.38212/2224-6614.2024.

- 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, Switzerland: Humana Press. DOI: 10.1007/978-3-030-05315-4\_14.
- Pignitter, M., Stolze, K., Gartner, S., Dumhart, B., Stoll, C., Steiger, G. et al. (2014): Cold fluorescent light as major inducer of lipid oxidation in soybean oil stored at household conditions for eight weeks. In: *Journal of agricultural and food chemistry* 62 (10), pp. 2297–2305. DOI: 10.1021/jf405736j.
- Poljsak, B., Kovač, V., Milisav, I. (2021): Antioxidants, Food Processing and Health. In: *Antioxidants (Basel, Switzerland)* 10 (3). DOI: 10.3390/antiox10030433.
- Poore, J., Nemecek, T. (2018): Reducing food's environmental impacts through producers and consumers. In: *Science (New York, N.Y.)* 360 (6392), pp. 987–992. DOI: 10.1126/science.aag0216.
- Rabadán, A., Pardo, J. E., Gómez, R., Álvarez-Ortí, M. (2018): Influence of temperature in the extraction of nut oils by means of screw pressing. In: *LWT - Food Science and Technology* 93, pp. 354–361. DOI: 10.1016/j.lwt.2018.03.061.
- Rabiej-Kozioł, D., Momot-Ruppert, M., Stawicka, B., Szydłowska-Czerniak, A., (2023): Health Benefits, Antioxidant Activity, and Sensory Attributes of Selected Cold-Pressed Oils. In: *Molecules (Basel, Switzerland)* 28 (14). DOI: 10.3390/molecules28145484.
- Ranstam, J., Cook, J. A. (2018): LASSO regression. In: *British Journal of Surgery* 105 (10), p. 1348. DOI: 10.1002/bjs.10895.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999): Antioxidant activity applying an improved ABTS radical cation decolorization assay. In: *Free radical biology & medicine* 26 (9-10), pp. 1231–1237. DOI: 10.1016/S0891-5849(98)00315-3.
- Rezig, L., Martine, L., Nury, T., Msaada, K., Mahfoudhi, N., Ghzaïel, I. et al. (2022): Profiles of Fatty Acids, Polyphenols, Sterols, and Tocopherols and Scavenging Property of Mediterranean Oils: New Sources of Dietary Nutrients for the Prevention of Age-related Diseases. In: *Journal of oleo science* 71 (8), pp. 1117–1133. DOI: 10.5650/jos.ess22110.
- Rhee, S. G. (1999): Redox signaling: hydrogen peroxide as intracellular messenger. In: *Experimental & molecular medicine* 31 (2), pp. 53–59. DOI: 10.1038/emm.1999.9.
- Rodilla, S. D., Martínez-Pineda, M., Yagüe-Ruiz, C., Vercet, A. (2023): Evaluation of phenolic compounds, antioxidant activity and pigment content in emerging and traditional plant-based oils in Mediterranean gastronomy. In: *International Journal of Gastronomy and Food Science* 33, p. 100771. DOI: 10.1016/j.ijgfs.2023.100771.

- Rombaut, N., Savoie, R., Thomasset, B., Castello, J., van Hecke, E., Lanoisellé, J.L. (2015): Optimization of oil yield and oil total phenolic content during grape seed cold screw pressing. In: *Industrial Crops and Products* 63, pp. 26–33. DOI: 10.1016/j.indcrop.2014.10.001.
- Savoie, R., Lanoisellé, J.L., Vorobiev, E. (2013): Mechanical Continuous Oil Expression from Oilseeds: A Review. In: *Food Bioprocess Technol* 6 (1), pp. 1–16. DOI: 10.1007/s11947-012-0947-x.
- Schwab, K. (2016): The fourth industrial revolution (1st ed.) Geneva, Switzerland: World Economic Forum. Available at: <https://permalink.obvsg.at/AC15429929>.
- Serazetdinova, L., Garratt, J., Baylis, A., Stergiadis, S., Collison, M., Davis, S. (2019): How should we turn data into decisions in AgriFood? In: *Journal of the science of food and agriculture* 99 (7), pp. 3213–3219. DOI: 10.1002/jsfa.9545.
- Singleton, V. L., Rossi, Joseph A. (1965): Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. In: *Am J Enol Vitic.* 16 (3), pp. 144–158. DOI: 10.5344/ajev.1965.16.3.144.
- Taib, NA. A. B., Rahman, M. R., Huda, D., Kuok, K. K., Hamdan, S., Bakri, M. K. B. et al. (2023): A review on poly lactic acid (PLA) as a biodegradable polymer. In: *Polym. Bull.* 80 (2), pp. 1179–1213. DOI: 10.1007/s00289-022-04160-y.
- Velasco, J., Dobarganes, C. (2002): Oxidative stability of virgin olive oil. In: *Eur. J. Lipid Sci. Technol.* 104 (9-10), pp. 661–676. DOI: 10.1002/1438-9312(200210)104:9/10<661::AID-EJLT661>3.0.CO;2-D.
- Vukic, M., Vujasinovic, V. (2023): Minor Bioactive Compounds of Cold-pressed Oils and Their Biomedical Functions: a Brief Review. In: *Int. J. Biomed. Healthc.* 11 (4), p. 364. DOI: 10.5455/ijbh.2023.11.364-370.
- Wang, B., Wang, S., Wang, Y., Zhang, S., Lin, X., Xu, X. et al. (2023): Deep exploration of lipid oxidation into flavor compounds: A density functional theory study on (E)-2-decenal thermal oxidative reaction. In: *Food Chemistry* 428, p. 136725. DOI: 10.1016/j.foodchem.2023.136725.
- Wen, C., Shen, M., Liu, G., Liu, X., Liang, L., Li, Y. et al. (2023): Edible vegetable oils from oil crops: Preparation, refining, authenticity identification and application. In: *Process Biochemistry* 124, pp. 168–179. DOI: 10.1016/j.procbio.2022.11.017.
- Wheeler, D. H. (1932): Peroxide formation as a measure of autoxidative deterioration. In: *J Americ Oil Chem Soc* 9 (4), pp. 89–97. DOI: 10.1007/BF02553782.

Whitfield, S., Challinor, A. J., Rees, R. M. (2018): Corrigendum: Frontiers in Climate Smart Food Systems: Outlining the Research Space. In: *Front. Sustain. Food Syst.* 2, Article 75. DOI: 10.3389/fsufs.2018.00075.

World Health Organization. (2023). World health statistics 2023: Monitoring health for the SDGs, sustainable development goals (1st ed.). Geneva, Switzerland: WHO. Available at: <https://ebookcentral.proquest.com/lib/kxp/detail.action?docID=31062296>.

Zhang, W., Huang, H., Wang, L., Lehmann, B. D., Chen, S. X. (2025): An Integrative Multi-Omics Random Forest Framework for Robust Biomarker Discovery. In: *bioRxiv : the preprint server for biology*. DOI: 10.1101/2025.03.05.641533.

Zhou, Z., Qiu, C., Zhang, Y. (2023): A comparative analysis of linear regression, neural networks and random forest regression for predicting air ozone employing soft sensor models. In: *Scientific reports* 13 (1), p. 22420. DOI: 10.1038/s41598-023-49899-0.



## LIST OF ABBREVIATIONS

| Abbreviation       | Meaning                                                |
|--------------------|--------------------------------------------------------|
| <sup>1</sup> H NMR | Proton nuclear magnetic resonance                      |
| µg                 | Micro gram                                             |
| ABTS               | 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) |
| ALA                | α-Linolenic acid                                       |
| ANOVA              | Analysis of variance                                   |
| AV                 | Acid value                                             |
| BC                 | Black cumin oil                                        |
| CLR                | Centered log ratio                                     |
| DAG                | Diacylglycerol                                         |
| DPPH               | 2,2-Diphenyl-1-picrylhydrazyl assay                    |
| ESI                | Electrospray ionization                                |
| FA                 | Fatty acid                                             |
| FAME               | Fatty acid methyl ester                                |
| FFA                | Free fatty acid                                        |
| FRAP               | Ferric reducing antioxidant power                      |
| GC-FID             | Gas chromatography - flame ionization detector         |
| GC-MS              | Gas chromatography - mass spectrometry                 |
| HME                | Heptadecanoic methyl ester                             |
| HO                 | High-oleic                                             |
| HPLC               | High-performance liquid chromatography                 |
| HS                 | Hempseed oil                                           |
| LASSO              | Least Absolute Shrinkage and Selection Operator        |
| LC-MS              | Liquid chromatography - mass spectrometry              |
| LOD                | Limit of detection                                     |
| LOQ                | Limit of quantification                                |
| LOX                | Lipoxygenase                                           |
| LS                 | Linseed oil                                            |
| MAG                | Monoacylglycerol                                       |
| mg                 | Milli gram                                             |
| mL                 | Milli litres                                           |
| ML                 | Machine learning                                       |
| MRM                | Multiple-Reaction-Monitoring                           |
| MUFA               | Monounsaturated fatty acid                             |
| NIAS               | Non-intentionally added substances                     |
| NIST               | National Institute of Standards and Technology         |
| OIT                | Oxidative induction time                               |
| PCA                | Principal Component Analysis                           |
| PE(16:0/18:1)      | 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine  |
| PET                | Polyethylene terephthalate                             |

|      |                                             |
|------|---------------------------------------------|
| PUFA | Polyunsaturated fatty acid                  |
| PV   | Peroxide value                              |
| PVDF | Polyvinylidene fluoride                     |
| QqQ  | Triple quadrupole mass spectrometer         |
| QTOF | Quadrupole time-of-flight mass spectrometer |
| RFR  | Random Forest regression                    |
| ROS  | Reactive oxygen species                     |
| RS   | Rapeseed oil                                |
| SD   | Standard deviation                          |
| SF   | Sunflower oil                               |
| SFA  | Saturated fatty acid                        |
| SPME | Solid phase micro extraction                |
| TAG  | Triacylglycerol                             |
| TPC  | Total polyphenol content                    |
| UV   | Ultra violett                               |

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## APPENDIX. CURRICULUM VITAE

### Education

2022-2025: (ongoing): PhD in Chemistry

at the working group “Analytical Food Chemistry & Food Development” at the Institute of Physiological Chemistry, Faculty of Chemistry, University of Vienna

Dissertation: „Novel Strategies and Predictive Models to Enhance Food Quality“

2019-2021: Master of Science (MSc)

In Food Technology and Nutrition at the University of Applied Sciences Upper Austria, Wels, Exchange Semester at Eesti Maaülikool University EMÜ, Tartu, Estonia

2016-2019: Bachelor of Science (BSc)

In Food Technology and Nutrition at the University of Applied Sciences Upper Austria, Wels, Exchange Semester at Pukyong National University PKNÜ, Busan, South Korea

2010-2015: HTL for Electronics (Telecommunications) in Leonding

### Professional Career

2021-2025: Doctoral Researcher

working group “Analytical Food Chemistry & Food Development” at the Institute of Physiological Chemistry, Faculty of Chemistry, University of Vienna

2021-2022: Research Associate

working group “Computed Tomography” at University of Applied Sciences Upper Austria, Wels

Feb-Jul 2021: Master’s internship at “Computed Tomography” research group

Oct-Feb 2019-2020: Laboratory Assistance at Brau Union Österreich

Apr-Jun 2019: Product Developer at Haribo Linz

### Publications

Pointner, T., Steinlechner, P., Pignitter, M. (2025). Fatty Acids, Polyphenols and Volatiles as Predictive Biomarkers of Cold-Pressed Oil Stability. *Manuscript currently under review in Food Chemistry: X*

Pointner, T., Rauh, K., Auñon-Lopez, A., Pignitter, M. (2025). Enhancing nutritional quality and oxidative stability in cloudy linseed oil through green extraction from press cake. *LWT*, 227,118034. DOI: 10.1016/j.lwt.2025.118034.

Pointner, T., Rauh, K., Auñon-Lopez, A., Kostadinović Veličkovska, S., Mitrev, S., Arsov, E. and Pignitter, M. (2024). Comprehensive analysis of oxidative stability and nutritional values of germinated linseed and sunflower seed oil. *Food Chemistry*, 454, 139790. DOI: 10.1016/j.foodchem.2024.139790

Pointner, T., Auer, J., Senck, S., Kastner, J., Mathmann, K. (2022). Comparison of preparation methods for the structural examination of meat using micro-computed tomography. *e-Journal of Nondestructive Testing*, 27(3). DOI: 10.58286/26624

Auñon-Lopez, A., Rial, C., Pointner, T., & Pignitter, M. Temperature-dependent antioxidative effects of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine fortification in polyunsaturated fatty acids-rich vegetable oils. *Manuscript currently under review in LWT*

Holler, M., Alberdi-Cedeño, J., Auñon-Lopez, A., Pointner, T., Martínez-Yusta, A., König, J. and Pignitter, M. (2023). Polylactic acid as a promising sustainable plastic packaging for edible oils. *Food Packaging and Shelf Life*, 36, 101051. DOI: 10.1016/j.fpsl.2023.101051

## Presentations

Oral:

- Approaches to Oil Stability: Bioactives, Biomarkers and Prediction Models, Joint Retreat Food quality & (non-) flavor activity, Wien, Austria, 09.10.2025
- Linseed Oil from Germinated Seeds Revealed Enhanced Nutritional Quality, Oxidative Stability and Bioactivity, AOCS Annual Meeting and Expo, Montreal, Canada, 28.04.2024
- Maximizing the Value of Aronia and Elderberry Pomace Through Particle Size Precision for Enhanced Polyphenol Bioaccessibility, Food Chemistry Days 2024, Wien, Austria, 03.04.2024
- Comprehensive Analysis of Oxidative Stability and Nutritional Values of Germinated Linseed Oils, 3<sup>rd</sup> Food Chemistry Conference, Dresden, Germany, 10.10.2023

- Analyse des Einflusses der Keimung auf Qualitätsparameter und die Haltbarkeit von Leinöl und Sonnenblumenöl, 77. ALVA – Jahrestagung, Linz, Austria, 22.05.2023
- Comparison of preparation methods for the structural examination of meat using micro-computed tomography, 11th Conference on Industrial Computed Tomography (iCT) 2022, 8-11 Feb, Wels, Austria

#### Poster:

- Comprehensive Fingerprinting and Machine Learning-Driven Scanning for Shelf-Life Prediction of Cold-Pressed Oils, Future Foods Congress, Singapore, 17.06.2025
- Lipidomic and Metabolomic Insights from Supplementation with Linseed Oil from Germinated Seeds, 5<sup>th</sup> DoSChem Symposium, Wien, Austria, 26.09.2025
- Lipidomics Reveals Benefits of Germinated Linseed Oil: From Enhanced Nutritional Quality and Oxidative Stability to Tissue-Specific Remodeling, Lipidomics Forum, Wien, Austria, 14.09.2025
- Cloudy Linseed Oil: New Strategies to Improve Stability and Nutritional Value , 4<sup>th</sup> International DoSChem Retreat Panel B, Rőjtökmuzsaj, Hungary, 25.04.2024
- Enhanced oxidative stability and antioxidant capacity of germinated linseed and sunflower oils, 3<sup>rd</sup> Food Chemistry Conference, Dresden, Germany, 10.10.2023
- Polyactic acid as a promising sustainable plastic packaging for edible oils, 3<sup>rd</sup> DoSChem Retreat, Panel B, Fürstenfeld, Austria, 20.04.2023

#### Awards

- Best Early Career Researcher Poster Award, Future Foods, 2025
- DoSChem New Ideas Programme Award 2025
- DoSChem Poster Award 2025
- Student Travel Grant Award, AOCS, Montreal, Canada, 2024
- DoSChem International Exchange Programme Award 2024