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Impact of Extraction Methods and Storage on the (Poly)Phenol and Fatty Acid Profiles of Walnut and Flaxseed Oils

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Abstract

While extensive research has been conducted on oil extraction methods, few studies have explored the variation in (poly)phenol profiles of vegetable oils over storage periods. This study investigated the impact of different industrial-scale extraction methods on the phytochemical composition of walnut (Juglans regia L.) and flaxseed (Linum usitatissimum L.) oils, focusing on changes in (poly)phenol content and fatty acid profiles at the initial time point and after eight months of storage. Two extraction techniques were evaluated: an Archimedes screw press and a prototype hydraulic piston press. Comprehensive analysis was conducted using RP-HPLC-DAD to quantify (poly)phenols and GC-FID to analyze fatty acids. Initial results revealed significant (poly)phenol content in both oils, with walnut oil containing 359 µg/kg and flaxseed oil 507 µg/kg when extracted using the Archimedes screw press, compared to 125 µg/kg in walnut oil and 482 µg/kg in flaxseed oil extracted with the prototype hydraulic piston press. Overall, the extraction methods and storage period had minimal impact on the fatty acids. These results have practical significance for the oil industry, highlighting the need to choose suitable extraction methods to improve the bioactive properties of cold-pressed oils.

Keywords: Fatty Acids, GC-FID, HPLC-DAD, Oils, (Poly)phenols, Storage

1.INTRODUCTION

In recent decades, the global demand for high-quality vegetable oils has grown exponentially, driven not only by their culinary applications but also by their emerging role in cosmetics, pharmaceuticals, and nutraceuticals. Walnut oil (Juglans regia L.) and flaxseed oil (Linum usitatissimum L.) have gained popularity as niche products. Highly valued for their rich nutritional content and bioactive compounds, including polyunsaturated fatty acids (PUFAs) such as omega-3 and omega-6, and (poly)phenols, walnut oil and flaxseed oil have seen a steady increase in market demand and price (Al-Madhagy et al., 2023; Gao et al., 2024; H. Song et al., 2022; Yang et al., 2021). Their versatility as valuable ingredients in both dietary and skincare products continues to drive consumer interest and industry innovation. However, the quality and quantity of these bioactive compounds are highly sensitive to the extraction method employed.

The (poly)phenolic profile of walnut oil is primarily characterized by tannins, including glansreginin B, and phenolic acids both known for their potent antioxidant activity. These compounds not only protect the oil from oxidative damage but also play a crucial role in human health by reducing oxidative stress and inflammation (Vivarelli et al., 2023; H. Zhang & Tsao, 2016). Similarly, flaxseed oil is distinguished by its high lignan content, alongside phenolic acids such as vanillic acid and ferulic acid, which

contribute to its antioxidative and health-promoting properties (Herchi et al., 2011).

From a fatty acid perspective, walnut oil is rich in alpha-linolenic acid (ALA), linoleic acid, and oleic acid, offering a favorable omega-6 to omega-3 ratio beneficial for cardiovascular health (Gharibzahedi et al., 2014; J.-J. Zhang et al., 2023). On the other hand, flaxseed oil stands out for its high ALA content, ranging from 40% to 60%, making it one of the most concentrated plant-based sources of omega-3 fatty acids (Yang et al., 2021).

Traditionally, oil extraction methods have included solvent extraction and mechanical pressing, with cold pressing gaining prominence for preserving heat-sensitive bioactive molecules. The choice of extraction method directly influences the oil's fatty acid profile, the retention of (poly)phenols, and overall nutritional quality (Fathollahi et al., 2021; L. Song et al., 2023; Van Hoed et al., 2010; Yilmaz & Güneşer, 2017). While solvent extraction is recognized for higher yields, it often compromises the bioactivity and purity of the final product due to chemical residues and thermal degradation. Conversely, cold-pressing methods, including mechanical screw presses and hydraulic piston presses, are celebrated for maintaining the integrity of bioactive compounds but are associated with variable yields and operational challenges.

Despite extensive research on individual extraction techniques, comparative analyses of the effects of various cold-pressing methods on the (poly)phenolic and lipid profiles of oils from non-common seeds remain limited and underexplored. This gap persists, particularly in understanding how specific operational parameters—such as rotational speed in screw presses and applied pressure in hydraulic presses—affect the concentration and stability of key bioactive compounds. Furthermore, the role of post-extraction storage conditions in preserving these compounds over time warrants further investigation, as oxidative degradation remains a critical concern for oils rich in unsaturated fatty acids.

This study seeks to address these knowledge gaps by comparing two cold-pressing extraction systems: the Archimedean screw expeller and a prototype hydraulic piston press "GSR". Through controlled variation of operational parameters—rotational speed for the expeller and applied pressure for the piston press—the study evaluates their impact on the composition of (poly)phenols and fatty acids in walnut oil and flaxseed oil. Additionally, the research examines the long-term stability of these compounds during storage under dark conditions for eight months, simulating real-world supply chain storage scenarios.

The outcomes of this research are anticipated to provide valuable insights into optimizing extraction methodologies to maximize the retention of bioactive compounds while ensuring product stability and quality. Such findings are not only academically significant but also have practical implications for the oil extraction industry, supporting the development of sustainable and efficient processing techniques that align with consumer demand for high-quality, nutrient-rich oils.

2. MATERIALS AND METHODS

2.1. Samples

In this study, cold-pressed walnut (N) and flaxseed (F) oils were analyzed. The oil samples were sourced from the oil mill F.lli Ruata S.P.A. (Baldissero d'Alba, Cuneo, Italy). Cold pressing was performed using two distinct types of presses: an Archimedes "Expeller" screw press (E) and a prototype "GSR" hydraulic piston press (G).

For each press type, two different operating conditions were applied, and the resulting oil temperatures were recorded (Table 1). The Archimedes "Expeller" screw press was operated at two rotational speeds: 70% and 100% of its maximum speed. Meanwhile, the prototype GSR operated at two pressure

settings: 860 kg/cm² for 700 seconds and 900 kg/cm² for 900 seconds.

Table 1. Samples, presses used, and extraction condition parameters provided by the manufacturer.

Oil	Press	Condition	Speed/Pressure	Outcome temperature
Walnut	Expeller (E)	1	70%	52 °C
		2	100%	50 °C
	GSR (G)	1	860 kg/cm2 – 700″	55 °C
		2	900 kg/cm2 – 900″	50 °C
Flaxseed	Expeller (E)	1	70%	72 °C
		2	100%	70 °C
	GSR (G)	1	860 kg/cm2 – 700″	72 °C
		2	900 kg/cm2 – 900″	70 °C

Each oil extraction was performed in duplicate. The resulting oil samples were stored in dark glass bottles and analyzed at two time points: the initial time point (t0) and after 8 months (t1) to evaluate changes in quality following storage.

2.2. Chemicals

Methanol (HPLC grade) and formic acid (50%, LC–MS grade) were purchased from Carlo Erba (Milan, Italy). Ultrapure water was obtained by Milli-Q instrument (Millipore Corp., Bedford, MA, USA). All the other chemicals, solvents and standards (purity ≥ 96 %, HPLC grade) were purchased from Sigma–Aldrich (Milan, Italy).

2.3. Phenolic compounds extraction

Polar (poly)phenols were extracted from walnut and flaxseed oils following the method described by Romani et al., 2017. Briefly, 19 g of the lipid sample was mixed with 19 mL of ethanol (70% v/v), acidified with formic acid (pH = 2), in a 50 mL centrifuge tube. The mixture was vortexed for 1 min at room temperature, incubated on an orbital undulating shaker (Sunflower 3D Mini-Shaker, BioSan, Latvia) for 30 min, and subsequently centrifuged at 5500 rpm for 15 min (Centrifuge 5804R, Eppendorf, Italy). The resulting supernatant was collected. The extraction procedure was repeated twice under the same conditions, and the supernatants from all three extractions were combined. The pooled ethanolic extract was washed three times with 35 mL of hexane in a separating funnel to remove residual oils. The purified extract was then dried at 42°C using a rotary evaporator (Rotavapor® R-210, Büchi, Switzerland). The resulting dry residue was re-dissolved in 3 mL of ethanol (70% v/v), filtered through a 0.45 µm nylon syringe filter, and stored at -18 °C until further analysis.

2.3.1. RP-HPLC-DAD analysis

The qualitative and quantitative analysis of the (poly)phenolic profile of walnut seed and flaxseed oils was performed using a Shimadzu

LC-20A Prominence HPLC system equipped with a diode array detector (SPD-M20A) and an autosampler (SIL-20A). Separation was achieved on a reversed-phase Luna C18 column (150 x 2 mm i.d., 5 μm particle size) (Phenomenex, Torrance, CA, USA), maintained at 30 °C, following the chromatographic method described by Giordano et al. (2017) with slight modifications.

The mobile phase consisted of ultrapure water acidified with 0.1% (v/v) formic acid (eluent A) and methanol acidified with 0.1% (v/v) formic acid (eluent B). The gradient applied was: from 5 to 17.5% B (0-30 min), from 17.5 to 30% B (10 min), from 30 to 100% B (5 min), isocratic 100% B (10 min), from 100 to 5% B (1 min), and finally isocratic 5% B to equilibrate the column (19 min).

The total run time was 75 min at a constant flow rate of 0.4 mL/min, with an injection volume of 7 μL.

Tentative identification of compounds was achieved by comparing retention times and UV-Vis spectra with those of authentic standards at 330 nm and at 280 nm. Final results are expressed as μg of analyte per kg of oil.

2.4. Identification and quantification of fatty acids by GC-FID

To determine the fatty acid profile, the samples were subjected to fatty acid methyl ester (FAME) derivatization according to the method of Locatelli et al., 2011. Subsequent analysis by gas chromatography with flame ionization (GC-FID) enabled the separation and quantification of the individual fatty acids as relative percentage.

For transesterification, 200 μL of oil sample was pipetted into a 2 mL glass vial. Subsequently, 200 μL of 0.5 N sodium methoxide in methanol was added. The vials were then incubated in a thermomixer (eppendorf thermomixer comfort, Eppendorf SE, Hamburg, Germania) at 80 °C and 350 rpm for 30 minutes. After cooling, 250 μL of deionized water and 500 μL of diethyl ether were added to each vial. Following vigorous vortexing and phase separation, 50 μL of the upper ether phase was transferred to a new vial containing 950 μL of dichloromethane.

Gas chromatographic analysis was conducted using a Thermo Trace 1300 gas chromatograph (Thermo Fisher Scientific, Waltham, Massachusetts) equipped with a split/spitless injector. Separation was achieved on a DB-23 J&W Scientific (Supelco) capillary column (30 m x 0.25 mm, 0.25 μm film thickness) using hydrogen (H2) as the carrier gas at a flow rate of 1.5 mL/min and a split ratio of 50:1. The injector and detector temperatures were set at 250 °C and 350 °C, respectively. A temperature program with a 5 °C/min ramp was employed. Identification of FAMEs was performed by comparing their retention times to those of a Supelco 37 Component FAME mix standard.

2.5. Statistical analysis

All the statistical analysis were performed using the statistical software R 4.2.1 (Boston, USA). Results were expressed as mean ± standard deviation (SD). Differences were assessed by analysis of variance (ANOVA) followed by Tukey's honest significant difference test and the statistical significance level was set to 0.05.

3. RESULTS AND DISCUSSION

3.1. Phenolic compounds

3.1.1. Walnut oil

This section presents the results of RP-HPLC-DAD analysis, summarizing the main (poly)phenolic compounds identified in walnut oil at t0 and t1 (after eight months of storage) obtained using the Archimedes “Expeller” screw press (E) and a prototype “GSR” hydraulic piston press (G). Table 2 shows the concentrations of these compounds

Table 2. (Poly)phenol content in walnut oil at t0 and t1

Compounds (µg/kg)	Calibration curve	R^2	Time	E1	E2	G1	G2
Protocatechuic acid	y = 3883x – 213.82	0.997	t0	118±8^{a}	117±6^{a}	18.8±2.0^{bA}	19.8±1.1^{b}
			t1	119±4^{a}	112±7^{a}	12.3±1.7^{aB}	22.3±2.3^{b}
p-Hydroxybenzoic acid	y = 2081.3x + 114.67	0.990	t0	48.5±1.7^{B}	47.0±3.8^{B}	nd	nd
			t1	58.3±2.3^{bA}	63.2±3.1^{aA}	nd	nd
Glansreginin B	y = 9907.4x – 1078.9	0.998	t0	75.0±0.6	75.0±1.3	nd	nd
			t1	76.9±2.6	78.9±1.6	nd	nd
Vanillic acid	y = 4564.6x + 70.976	0.994	t0	6.37±0.40^{B}	6.46±0.10^{B}	nd	nd
			t1	8.86±0.70^{A}	8.03±0.90^{A}	nd	nd
Chlorogenic acid	y = 10246x – 7.8462	0.986	t0	19.1±3.6	17.4±2.0	nd	nd
			t1	19.1±0.2	19.3±1.5	nd	nd
Coumaric acid	y = 10425x + 203.2	0.998	t0	24.0±1.6^{b}	24.0±1.5^{b}	28.2±0.7^{a}	27.9±2.7^{a}
			t1	24.1±1.4^{b}	25.1±0.1^{b}	28.2±1.3^{a}	28.2±0.3^{a}
Ellagic acid	y = 9907.4x – 1078.9	0.998	t0	68.5±1.3^{B}	68.7±1.0^{B}	78.3±5.9^{A}	68.9±7.1^{A}
			t1	108±8^{aA}	101±4^{aA}	42.7±7.7^{bB}	41.7±4.5^{bB}

Values are expressed as mean ± standard deviation (µg/kg oil). Within each row, different lowercase letters indicate significant differences ($p < 0.05$) between samples, while uppercase letters indicate significant differences between t0 and t1 for each phenolic compound. Values without letters are not significantly different. Statistical differences were assessed by ANOVA followed by Tukey's HSD test. nd: not detectable, E = Expeller Screw Press; G = GSR hydraulic piston press.

At time t0, the highest phenolic content was obtained by pressing walnuts using the expeller screw press, yielding 359.4 µg/kg and 355.5 µg/kg under mild (E1) and higher rotational speeds (E2), respectively. In contrast, the GSR hydraulic piston press produced lower phenolic concentrations, with values of 125.3 µg/kg when a force of 860 kg/cm² was applied for 700 s (G1), and 116.6 µg/kg when a force of 900 kg/cm² was applied for 900 s (G2). According to Ojeda-Amador et al. (2018),
the concentration of phenolic compounds in commercial walnut oil ranges from 210 to 10,600 µg/kg.

However, a comprehensive comparison with other findings is challenging, as, to the best of our knowledge, there is a lack of studies characterizing these polar compounds in detail. RP-HPLC-DAD analysis revealed the presence of seven (poly)phenols, predominantly phenolic acids. The major phenolic compound detected is protocatechuic acid, using Archimede expeller screw press, the amount of this compound amounts at 118 (E1) and 117 µg/kg (E2) at t0. While using the GSR hydraulic piston press the levels were significantly lower, amounting at 18 µg/kg for G1 and 19 µg/kg for G2

condition, approximately 10 times lower compared whit Archimede expeller screw.

Glansreginin B levels, at t0, were consistently high in all extractions performed using the Archimede expeller screw press. In both E1 and E2 conditions, glansreginin B levels were found to be 75 µg/kg. Unexpectedly, no glansreginin B was detected in any samples extracted using the GSR hydraulic piston press. Chemically, glansreginin B is a dicarboxylic acid derivative that was newly isolated from walnuts by Hideyuki et al. The authors elucidated its structure using high-resolution electrospray ionization mass spectrometry (HRESIMS), as well as 1H and 13C NMR, identifying the presence of a glansreginic acid moiety and a sucrose unit (Ito et al., 2007). Our findings were in accordance to those of Ojeda-Amador et al., which confirmed the presence of glansreginin B and A in walnut oil. The authors reported the presence of glansreginin B in three distinct varieties of walnuts at concentrations between 10 and 170 µg/kg (Ojeda-Amador et al., 2018). Slatnar et al. investigated the presence of glansreginin B in oils extracted from five different walnut varieties (Slatnar et al., 2015). The highest concentration of glansreginin B was observed in oil derived from the 'Fernor' cultivar, reaching 53 µg/kg, while the lowest value was found in oil from the 'Franquette' cultivar at 8 µg/kg.

Similar trends were observed for p-hydroxybenzoic, vanillic, and chlorogenic acids which were not detected in G extractions. p-Hydroxybenzoic acid levels were 48.5 µg/kg in E1 and 47.0 µg/kg in E2 while vanillic acid levels were 6.37 µg/kg in E1 and 6.46 µg/kg in E2 at t0. The presence of vanillic acid is confirmed by Al Juhaimi and colleagues, which detected 1.8 µg/kg of vanillic acid in walnuts oil extracted with cold press and 2.9 µg/kg in oil extracted using the Soxhlet method (Al Juhaimi et al., 2018). The same authors also confirmed the presence of chlorogenic acid at concentrations ranging from 6.1 to 6.7 µg/kg, which are lower than those quantified in our samples (19.1 µg/kg and 17.4 µg/kg in E1 and E2, respectively).

In G1 condition, the level of coumaric acids amount at 28.2 µg/kg and 27.9 µg/kg in G2, and significantly lower in E1 (24.0 µg/kg) and E2 (24.0 µg/kg) at t0.

Ellagic acid levels at t0 did not differ significantly between the four extraction conditions. In E1 and E2 conditions, ellagic acid levels were 68.5 µg/kg and 68.7 µg/kg, respectively, while in G1 and G2 conditions, the levels were 78.3 µg/kg and 68.9 µg/kg, respectively.

Compared to walnut oil, raw walnut kernels contain high levels of (poly)phenols, more than 5000 mg/kg, which are largely retained in the cake representing the cell-wall bound fraction (Huang et al., 2024).

During storage, the profile of phenolic compounds may change, either increasing due to the degradation of complex structures such as hydrolysable tannins or decreasing due to the susceptibility of certain small molecules to temperature or storage condition (Mousavi et al., 2021). In their study of olive oil storage, Mousavi et al. (2021) documented changes in the (poly)phenol profile over 18 and 36 months relative to the initial composition. Their findings indicate a rise in simple phenols (tyrosol and hydroxytyrosol) after 36 months, coupled with a decline in complex (poly)phenols, including oleuropein and oleacin. These results support the hypothesis that complex (poly)phenols are susceptible to oxidative and hydrolytic degradation during extended storage (H. Cao et al., 2021). In general, in our study, the trend of (poly)phenol passes from 359.5 µg/kg and 355.5 µg/kg in E1 and E2 condition respectively at t0 to 414.2 µg/kg in E1 and 407.5 µg/kg in E2 condition at t1. While for the G1 and G2 extraction condition, the level pass from 125.3 µg/kg and 116.6 µg/kg to 83.2 and 92.2 µg/kg at t0 and t1, respectively.

After t1, the amount of p-hydroxybenzoic acid, result increased compared to t0 in E1 and E2 extraction, while in G1 and G2 there wasn't

reliable. The level of p-hydroxybenzoic acid increased 20.8% in E1 and 34% in E2 extraction condition after eight months.

Glansreginin B levels remained stable at t1 in E1, G1, and G2. In contrast, slight increase of 5.3% was observed in glansreginin B in E2 extraction condition at t1. In general, vanillic acid levels exhibited an increasing trend after eight months in both E1 and E2 extraction conditions, plus 39.1% and 24.3% respectively. Conversely, no traces of vanillic acid were detected in G1 and G2 extraction conditions at t1.

Interesting things are founds in ellagic acid trend. In E1 and E2 extraction conditions, has been registered one increasing after eight months. In E1 condition the ellagic acid increase of 58% in t1. In E2 conditions the increased was 47% after storage period. In contrast, a decrease has been observed in oil obtained from G1 and G2 extraction condition between t0 and t1. -45.5% for G1 and -39.5% for G2 extraction condition. At t1, after eight months of storage, the content of protocatechuic acid remained unchanged in walnut oil extracts obtained under E1, E2, and G2 conditions. A reduction of -34% in protocatechuic acid levels was observed only in the G1 extraction condition after storage period.

No significant differences in chlorogenic acid and coumaric acid content were observed among the four extraction conditions at both t0 and t1.

3.1.2. Flaxseed oil

Table 3 presents the concentration of individual (poly)phenols in oils extracted under different conditions (E1, E2, G1, and G2) at time zero (t0) and after eight months of storage (t1).

Table 3: (Poly)phenol content in flaxseed oil at t0 and t1

Compounds (µg/kg)	Calibration curve	R^2	Time	E1	E2	G1	G2
Protocatechuic acid	y = 4021.1x – 557.48	0.999	t0	147 ± 38^{a}	167 ± 31^{a}	69.9 ± 9.9^{b}	87.4 ± 14.0^{bA}
			t1	156 ± 31^{b}	206 ± 15^{a}	74.8 ± 12.0^{c}	65.7 ± 11.0^{cB}
p-Hydroxybenzoic acid	y = 2726.2x – 617.39	0.994	t0	69.8 ± 7.0^{bB}	78.6 ± 10.0^{b}	93.4 ± 1.8^{aB}	98.0 ± 7.7^{a}
			t1	84.7 ± 7.0^{bA}	82.4 ± 6.5^{b}	101 ± 6^{aA}	112 ± 10^{a}
Vanillic acid	y = 5451.3x – 837.1	0.997	t0	62.1 ± 1.8^{bB}	64.6 ± 2.9^{bB}	122 ± 5^{a}	118 ± 0^{a}
			t1	93.8 ± 3.1^{bA}	86.8 ± 4.5^{bA}	121 ± 20^{a}	127 ± 10^{a}
Caffeic acid	y = 10246x – 7,8462	0.991	t0	7.91 ± 0.90^{abB}	8.99 ± 1.10^{aB}	6.38 ± 0.40^{bA}	6.52 ± 0.90^{b}
			t1	13.0 ± 1.6^{aA}	12.1 ± 1.5^{aA}	5.59 ± 0.60^{bB}	5.15 ± 1.00^{b}
Chlorogenic acid	y = 12767x – 953,05	0.995	t0	23.5 ± 4.5^{aA}	20.6 ± 2.5^{a}	7.11 ± 0.80^{B}	6.64 ± 0.40^{bB}
			t1	17.4 ± 1.8^{bB}	21.5 ± 1.3^{a}	15.9 ± 1.9^{bA}	11.3 ± 1.8^{cA}
Epicatechin	y = 1683,2x + 5,449	0.999	t0	74.6 ± 17.0^{a}	58.5 ± 4.5^{abB}	49.2 ± 9.0^{bB}	51.2 ± 8.3^{b}
			t1	64.5 ± 5.5^{ab}	76.6 ± 10.0^{aA}	77.9 ± 10.0^{aA}	58.5 ± 6.3^{a}
Coumaric acid	y = 9907.4x – 1078.9	0.998	t0	29.8 ± 1.6	28.1 ± 3.2^{B}	26.8 ± 1.4^{B}	26.6 ± 1.4
			t1	32.6 ± 1.3^{ab}	33.6 ± 0.2^{aA}	31.4 ± 1.8^{abA}	29.0 ± 4.4^{b}
Ferulic acid	y = 10434x + 183,27	0.998	t0	55.1 ± 1.6^{bB}	57.3 ± 7.2^{abB}	66.6 ± 7.3^{aB}	67.0 ± 1.3^{aB}
			t1	87.7 ± 1.4^{A}	89.3 ± 0.7^{A}	105 ± 21^{A}	110 ± 26^{A}
Luteolin	y = 9421,2x – 2305	0.995	t0	24.6 ± 2.0^{a}	23.4 ± 2.4^{ab}	22.5 ± 1.4^{abA}	20.6 ± 1.2^{bA}
			t1	23.2 ± 0.5^{a}	23.0 ± 0.3	13.6 ± 1.4^{bB}	11.6 ± 1.4^{cB}

Values are expressed as mean ± standard deviation (µg/kg oil). Within each row, different lowercase letters indicate significant differences ($p < 0.05$) between samples, while uppercase letters indicate significant differences between t0 and t1 for each

phenolic compound. Values without letters are not significantly different. Statistical differences were assessed by ANOVA followed by Tukey's HSD test. nd: not detectable, E = Expeller Screw Press; G = GSR hydraulic piston press.

3.2. Fatty acids composition

3.2.1. Walnut oil

The results of the FAME analysis, conducted using GC-FID, are reported in Table 4 as relative percentages of fatty acid content.

Table 4. Fatty Acid Methyl Ester (FAME) Composition in walnuts oil

Fatty acid	Time	E1	E2	G1	G2
Palmitic acid (C16:0)	t0	6.61 ± 0.01^{B}	6.55 ± 0.03^{B}	6.53 ± 0.05^{B}	6.56 ± 0.01^{B}
	t1	7.19 ± 0.04^{A}	7.30 ± 0.11^{A}	7.06 ± 0.01^{A}	7.11 ± 0.07^{A}
Stearic acid (C18:0)	t0	2.66±0.01	2.67±0.02	2.60±0.02	2.60±0.03
	t1	2.64±0.01	2.68±0.05	2.61±0.02	2.65±0.08
Oleic acid (C18:1n9cis)	t0	19.9±0.0	19.8±0.0	20.0±0.0	20.1±0.1
	t1	19.6±0.0	19.7±0.0	19.9±0.4	19.8±0.3
Linoleic acid (C18:2n6cis)	t0	56.0 ± 0.1^{aB}	56.0 ± 0.0^{a}	55.7 ± 0.0^{bB}	55.8 ± 0.1^{ab}
	t1	56.3 ± 0.0^{aA}	56.1 ± 0.2^{ab}	55.9 ± 0.1^{abA}	55.6 ± 0.4^{b}
α-Linolenic acid (C18:3n3)	t0	13.5 ± 0.0^{bA}	13.5 ± 0.0^{bA}	13.9 ± 0.0^{aA}	13.8 ± 0.0^{ab}
	t1	12.5 ± 0.0^{B}	12.4 ± 0.0^{B}	12.9 ± 0.3^{B}	12.9±0.3

Fatty acid composition expressed as (%) ± standard deviation in walnut oil at t0 and t1. Within each row, different lowercase letters indicate significant differences ($p<0.05$) between samples, while uppercase letters indicate significant differences between t0 and t1 for each fatty acid. Values without letters are not significantly different. Statistical differences were assessed by ANOVA followed by Tukey's HSD test. nd: not detectable, E = Expeller Screw Press; G = GSR hydraulic piston press.

The analysis revealed a high prevalence of linoleic acid (55.6-56.3%), followed by oleic acid (19.6 to 20.1%) and α-linolenic acid (12.4 to 13.9%).

At t0, GC-FID analysis of FAMEs revealed no significant differences in fatty acid composition between walnuts oil extracts obtained using different technologies. The most notable differences, though minor, were observed in linoleic and α-linolenic acid content between extraction methods E and G.

In comparison with our results, Ghiasi et al., employing a cold extraction method, found no significant difference in fatty acid composition. Their analysis indicated the following composition: (8.2%) palmitic acid, (3.4%) stearic acid, (24%) oleic acid, and (51.6%) linoleic acid (Ghiasi et al., 2022). While Cao et al. reported palmitic acid (6.6%), stearic acid (2.3%), oleic acid (13.8%), linoleic acid (66%), and linolenic acid (11.1%) on walnut oil extracted using hydraulic pressing (S. Cao et al., 2024).

These results are in agreement with our data.

The ω6:ω3 ratio in walnut oil samples was found to be 4.5:1. This value aligns with nutritional guidelines recommending a ratio of ≤4:1 for a balanced and healthy diet, as an appropriate

balance of these fatty acids is essential for optimal physiological function. After eight months of storage, no significant differences were observed in stearic and oleic acid content. Palmitic acid levels increased in all samples: G1 increased of (8.9%), G2 of (11.5%), E1 (8.1%) and E2 (8.4%). Also linoleic acid has registered an significant increase in E1 and G1 oil (+0.5% and +0.4% respectively). These results contrast with those of Christopoulos & Tsantili (2012), who reported no increase in linoleic acid in cold-pressed walnut oil after one year of storage. They found that at t0, linoleic acid constituted 54% of the fatty acid, while after one year, represent 52% stored at 0 °C and 48% stored at 20 °C (Christopoulos & Tsantili, 2012).

Ampofo et al. studied the evolution of fatty acid composition in walnut oil during a four-month storage period. Their analysis revealed no significant variation in palmitic acid levels between the initial time point (2.3% at t_0) and after four months at 5 °C (2.3%). However, a decrease in palmitic acid concentration to 1.7% was observed after four months at room temperature. The authors reported no significant differences in the concentrations of α-linolenic, stearic, and oleic acids between t0 and after four months of storage at either 5°C or room temperature (Ampofo et al., 2022).

Conversely, the decrease in α-linolenic acid concentration observed after eight months of storage, (- 7.4%, -8.1%, -7.2% and -6.5% for E1, E2, G1 and G2 respectively) suggests oxidation of this fatty acid. This is further supported by the increase in peroxide value registered after six months, indicative of oxidative processes (Rébufa et al., 2022).

3.2.2. Flaxseed oil

Flaxseed oil, characterized by its unique fatty acid profile rich in unsaturated and polyunsaturated fatty acids, particularly α-linolenic acid, has garnered significant attention for its associated health benefits. Numerous studies have demonstrated that flaxseed oil consumption can exert anti- inflammatory effects and may contribute to the prevention of several non-communicable diseases, including cancer, atherosclerosis, and obesity, potentially through the downregulation of inflammatory genes (Yang et al., 2021). Thus, employing optimal extraction methods to prevent oxidation and maintain the quality of healthy fatty acids is of paramount importance.

The results of the FAME assay, conducted using GC-FID, are reported in this section (Table 5).

Table 5. Fatty Acid Methyl Ester (FAME) Composition in flaxseed oil

Fatty acid	Time	E1	E2	G1	G2
Palmitic acid (C16:0)	t0	6.07 ± 0.00^{B}	6.04 ± 0.00^{B}	6.05±0.05	6.03 ± 0.01^{B}
	t1	6.64 ± 0.06^{A}	6.74 ± 0.08^{A}	6.87±0.32	6.75 ± 0.03^{A}
Stearic acid (C18:0)	t0	4.39 ± 0.00^{B}	4.41±0.01	4.44±0.04	4.42±0.01
	t1	4.44 ± 0.08^{A}	4.53±0.05	4.49±0.02	4.45±0.03
Oleic acid (C18:1n9cis)	t0	16.3 ± 0.0^{aA}	16.3 ± 0.0^{a}	15.6 ± 0.0^{b}	15.6 ± 0.0^{bA}
	t1	16.1 ± 0.1^{B}	16.3±0.1	15.8±0.5	15.4 ± 0.0^{B}
Linoleic acid (C18:2n6cis)	t0	50.2 ± 0.0^{b}	49.9 ± 0.1^{b}	52.5 ± 0.1^{a}	52.4 ± 0.0^{a}
	t1	50.0 ± 0.1^{b}	50.0 ± 0.1^{b}	52.6 ± 0.0^{a}	52.4 ± 0.2^{a}
α-Linolenic acid (C18:3n3)	t0	21.6 ± 0.0^{aA}	21.6 ± 0.1^{aA}	20.0 ± 0.1^{bA}	20.1 ± 0.0^{bA}
	t1	20.4 ± 0.3^{aB}	20.3 ± 0.1^{abB}	18.2 ± 0.9^{bB}	18.9 ± 0.0^{B}

Fatty acid composition expressed as (%) ± standard deviation in flaxseed oil at t0 and t1. Within each row, different lowercase letters indicate significant differences ($p<0.05$) between samples, while uppercase letters indicate significant differences between t0 and t1 for each fatty acid. Values without letters are not significantly different. Statistical differences were assessed by ANOVA followed by Tukey's HSD test. nd: not detectable, E = Expeller Screw Press; G = GSR hydraulic piston press.

In our study, the primary fatty acids identified were linoleic acid and α-linolenic acid, which accounted for approximately 50% and 20% of the total concentration, respectively. These findings contrast with those of previous studies. For instance, Dedebas et al., 2021 and Gandova et al., 2023 reported α-linolenic acid concentrations of 57%, while Bera et al., (2006) observed 50%. Additionally, our study found a lower concentration of linoleic acid (50%) compared to the reports from Gandova et al. (2023) (12%), Bera et al. (2006) (14%), and Dedebas et al. (2021) (16.5%).

The concentrations of palmitic, stearic, and oleic acids observed in our study were consistent with those reported in other research (Gandova et al., 2023; Bera et al., 2006; Dedebas et al., 2021), with these fatty acids ranging approximately from 6%, 4%, and 20%, respectively.

Mikołajczak & Tańska (2022) highlighted that geographical origin and harvest conditions are key factors contributing to the variability in fatty acid composition, which may help explain the differences observed between our study and previous findings. Moreover, (Zhang et al., 2013) reported that prolonged exposure of flaxseed oil to elevated temperatures (>75 °C) induces alterations in its fatty acid profile, characterized by a decrease in α-linolenic acid and a concomitant increase in linoleic acid. According to the operational parameters provided by the oil mill, the outlet temperature of both pressing machines was approximately 70 °C (Table 1). These temperature conditions may have played a role in modulating the fatty acid composition of the extracted oil.

The analysis of linoleic acid and α-linolenic acid yields a nutritional ω6:ω3 ratio of 3:1, which contrasts with the ratio of 0.3:1 typically reported in the literature for this oil (Goyal et al., 2014).

At t0, GC-FID analysis of FAMEs showed minor variations in fatty acid composition among flaxseed oil extracts obtained using different technologies. While palmitic and stearic acid levels remained relatively consistent (approximately 6% and 4%, respectively) across all conditions, oleic and α- linolenic acids were more significantly higher in oils extracted from conditions E1 and E2 compared to G1 and G2. Linoleic acid, however, was more abundant in oils from conditions G1 and G2 (approximately 52%) than in E1 and E2 (approximately 50%).

Interesting study published by Kasote et al., (2013) that employed a screw expeller press to extract flaxseed oil, implementing single, double, and triple pressing protocols. Palmitic acid (5.6%) and linoleic acid (46%) levels remained stable across all three extraction conditions. Stearic acid levels decreased with successive extractions (6.2%, 5.7%, and 5.4%), whereas oleic acid levels increased (20.3%, 21.6%, and 24%) as well as for linolenic acid (11.4%, 12%, and 13.3%). The authors showed that the choice of extrusion method can substantially affect the yield and quality of the resulting oil (Kasote et al., 2013).

In comparison, the palmitic acid levels observed in our study are consistent with the findings reported by Kasote et al., (2013). However, our stearic and oleic acid levels were slightly lower, whereas our linoleic and linolenic acid levels were higher.

Following eight months of storage, increases in palmitic acid levels were observed in extracts obtained using methods E1, E2, and G2 (9.4%, 11.6%, and 11.9% increases, respectively). Stearic acid concentration increased only in the E1 extract (1.1% increase). Conversely, decreases in oleic acid concentration were noted in the E1 and G2 extracts (1.2% and 1.3% decreases, respectively), as were decreases in α-linolenic acid concentration in the E1, E2, and G2 extracts (5.1%, 6.5%, and 6% decreases, respectively). No significant change was observed in linoleic acid concentration.

The results of Dedabas et al., (2021) partially agree with our findings. During 12 months of storage at room temperature the authors observed notable changes in fatty acid composition. Saturated fatty acids increased, with palmitic acid rising from (5.6% to 6.5%), oleic acid from (17.6% to 20%), and stearic acid showing the most substantial increase, from 3.7% to 10.3%. In contrast, the polyunsaturated fatty acids linoleic acid and α-linolenic acid (likely intended) decreased, from 16.5% to 16.3% and 56.6% to 46%, respectively (Dedebas et al., 2021).

In contrast to our results, Prescha et al., (2014) found no significant changes in the fatty acid profile of flaxseed oil obtained from cold pressed technologies after 12 month of storage (Prescha et al., 2014). Similarly, Islam et al., (2023) reported no significant changes in the fatty acid composition of cold-pressed oil after 6 months of storage (Islam et al., 2023). Likewise, Malcolmson et al., (2000) reported no significant differences in fatty acid composition after 128 days of storage compared to initial levels (Malcolmson et al., 2000).

4. CONCLUSION

This study systematically examined the impact of two cold-pressing extraction methods—the Archimedean screw expeller and the GSR hydraulic piston press—on the (poly)phenolic and lipid composition of walnut and flaxseed oils. Additionally, the storage period led to varying trends in the stability of these bioactive compounds, with some phenolics increasing likely due to hydrolytic degradation of complex structures, while others declined, possibly as a result of oxidative processes. In walnut oil, the expeller press demonstrated superior retention of total and key (poly)phenols such as protocatechuic acid, glansreginin B, and vanillic acid, whereas the hydraulic press yielded significantly lower levels of these compounds. Conversely, in flaxseed oil, the total (poly)phenol content was comparable across extraction methods, with differences observed in the qualitative composition of individual compounds. Fatty acid composition remained largely stable across both extraction methods, though minor differences were observed in linoleic and α-linolenic acid levels. Storage led to a slight increase in saturated fatty acids and a decrease in α-linolenic acid, likely due to oxidative degradation.

Despite the valuable insights gained, certain limitations should be acknowledged. The study did not assess the sensory properties or oxidative markers of the oils, which could provide further clarity on quality deterioration over time. Additionally, while the research simulated real-world storage conditions, future studies should investigate the effects of different packaging materials and storage temperatures on oil stability. Further exploration into optimizing extraction parameters to maximize both yield and bioactive compound preservation is recommended.

These findings offer practical implications for the oil industry, emphasizing the importance of selecting appropriate extraction techniques to enhance the bioactive and functional quality of cold- pressed walnut and flaxseed oils.

DECLARATION OF CONFLICTING INTERESTS

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