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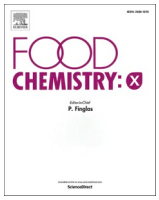

Unveiling the oxidative stability, phytochemical richness, and nutritional integrity of cold-pressed *Linum usitatissimum* oil under UV exposure

Oumayma Belhoussaine [a], Chaimae El Kourchi [a], Amakhmakh Mohammed [b], Adil EL Yadini [a], Riaz Ullah [c], Zafar Iqbal [d], Khang Wen Goh [e,f], Monica Gallo [g], Hicham Harhar [a], Abdelhakim Bouyahya [h,*], Mohamed Tabyaoui [a]

[a] *Laboratory of Materials, Nanotechnology and Environment, Faculty of Sciences, Mohammed V University in Rabat, Av. Ibn Battouta, B.P1014 Rabat, Morocco*
[b] *Laboratory of Applied Organic Chemistry, Faculty of Sciences and Technology, Sidi Mohamed ben Abdellah University in Fes, Morocco*
[c] *Department of Pharmacognosy College of Pharmacy King Saud University Riyadh, Saudi Arabia*
[d] *Department of Surgery, College of Medicine, King Saud University P.O.Box 7805, Riyadh 11472, Saudi Arabia*
[e] *Faculty of Data Science and Information Technology, INTI International University, Nilai, Malaysia*
[f] *Faculty of Engineering, Shinawatra University, Samkhok, Pathum Thani, Thailand*
[g] *Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Via Pansini, 5, 80131 Naples, Italy*
[h] *Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, Mohammed V University in Rabat, Morocco*

A R T I C L E I N F O

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A B S T R A C T

This study examines the effect of UV irradiation on the oxidation stability of *Linum usitatissimum* oil, presenting possible changes in the phytochemical profile due to photo-oxidation. GC–MS analysis of the oils identified 11 fatty acid compounds with a high percentage of unsaturated fatty acids, the most important of which is α-linolenic acid (ALA), known as omega-3 (48.88 %), also significant profiles of phytosterol and tcocopherol isomers rich in β-Sitosterol and γ-tocopherols respectively. As well as physicochemical properties such as free fatty acids (FFA %), peroxide value (PV) and iodine value (IV), and nutritional indexes that determine the significant changes observed during the oxidation process, the most important of which is the progressive increase in acidity, peroxide, conjugated dienes and trienes and degrees of unsaturation over 8 h of UV exposure. High levels of carotenoids and phenolic compounds (TPC) protect and enhance oil quality in the face of irradiation, so a significantly small difference is observed between irradiated and non-irradiated oil during photo-oxidation.

1. Introduction

Light radiation, temperature and atmospheric oxygen are factors that induce lipid oxidation and generate free radicals (Kchaou, Jridi, Nasri, & Debeaufort, 2020) leading to the formation of harmful primary oxidation products, such as peroxides, which then decompose into toxic secondary oxidation products, such as carbonyl compounds, conjugated dienes and furans (Rajeev, Sanjiv, & Mahender, 2012), as mentioned previously (Kato, 2008; Miraliakbari, Shahidi, & Chemistry, 2008) fats and oils are susceptible to photooxidation when exposed to UV light at wavelengths below 300 nm, contributing to the loss of nutritional and organoleptic properties of foods and also generating radical oxygen species that can cause irreversible damage when they react with biological molecules such as DNA, proteins or lipids (Choe & Min, 2006; Kchaou et al., 2020; Prescha, Grajzer, Dedyk, & Grajeta, 2014), this oxidative damage induces adverse effects on human health, such as the induction of metabolic diseases like diabetes and obesity (Zu-Man et al., 2024). The deterioration in the quality of some oils is caused mainly by

Abbreviations: LO, *Linum usitatissimum* oil; Control, Control *Linum usitatissimum* oil; 1H, 2H, 4H, 6H, 8H, Exposition time (hours); ∑SFA, Satured fatty acids; ∑MUFA, Monounsaturated fatty acids; ∑PUFA, Polyunsatured fatty acids; AI, Atherogenic index; TI, Thrombogenic index; Cox, Oxidazability index; OS, Oxidative susceptibility; ω3/ω6, Omega-3: Omega-6 ratio; TPC, Total polyphenols content; FFA, Free fatty acid; PV, Peroxide value; IV, Iodine valye; LDR, Linoleyl-desaturation ratios; ODR, Oleyl- desaturation ratios.

* Corresponding author.
E-mail addresses: mohammed.amakhmakh@um5r.ac.ma (A. Mohammed), a.elyadini@um5r.ac.ma (A. EL Yadini), rullah@ksu.edu.sa (R. Ullah), zsyed@ksu.edu.sa (Z. Iqbal), khangwen.goh@newinti.edu.my (K.W. Goh), mongallo@unina.it (M. Gallo), h.harhar@um5r.ac.ma (H. Harhar), a.bouyahya@um5r.ac.ma (A. Bouyahya), h.tabyaoui@um5r.ac.ma (M. Tabyaoui).

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their oxidation during storage and even the high sensitivity of polyunsaturated fatty acids, such as n-3 fatty acids (Frankel, 1984; Frueh-wirth et al., 2020) and Several compounds, such as hexanal or nonanal, are used as markers of the lipid oxidation process (Azarbad & Jeleń, 2015; Kalua et al., 2007).

Cold-pressed and refined oils differ in taste, aroma and colour, and are abundant in polyunsaturated fatty acids (PUFA), phenolic compounds, sterols and carotenoids, which are recommended for human consumption (Gunstone, 1994; Ramadan, 2013; Wei et al., 2015) Consequently, they should be consumed within 6 to 12 months of production (Frankel, 1984). Reviews summarize the qualitative properties of flaxseed oil for food use, presenting factors influencing quality and discussing the negative influence of oxidation on oil taste, colour and odour, reporting detailed agronomic, chemical, biotechnological and microbiological methods to determine the effects of storage on composition and methods to promote its quality (Nykter, Kymäläinen, Gates, & Science, 2006). Also the positive aspects for human health of polyunsaturated fatty acids, highlighting however their high sensitivity to oxidation, resulting in a loss of shelf life, consumer acceptability, functionality, nutritional value and safety (Arab-Tehrany et al., 2012). Then flaxseed oil, which is generally cold-pressed, necessarily stored in dark glass bottles (Łukaszewicz, Szopa, & Krasowska, 2004) and because of its short shelf life, it is often enriched with vitamins A and E or synthetic antioxidants (Hasiewicz-Derkacz et al., 2015; Łukaszewicz et al., 2004; Makahleh, Saad, & Bari, 2015).

Linum usitatissimum (Flaxseed) mainly consists of ω-3 fatty acids, sterols, antioxidants, phytoestrogenic lignans, phenols, flavonoids, proteins, as well as soluble and insoluble fibers, such as secoisolariciresinol (SDG) diglucosides are the main bioactive compounds with potential pharmacological realizations.(Akter et al., 2021). *L. usitatissimum* shows antioxidant, antimicrobial, anti-inflammatory, anticancer, antiobesity, antidiabetic, antimalarial, anti-diarrheal, hepatoprotective, immunosuppressive, antiarrhythmic, cognitively significant, and renoprotective effects. In addition, it is known as an analgesic, anti-fibrosis, blood sugar stabilizer, antiviral, antiatherosclerotic, and bactericide (Akter et al., 2021; Elrahman, Ahmed, Ibrahim, Seed, & Babiker, 2023).

In this study, cold-pressed flaxseed oil is exposed to UV irradiation, and samples are taken after specific exposure times. Irradiated and control oils are identified by their chemical compositions of fatty acids, phytosterols and tocopherols, and analyzed by nutritional and unsaturation indexes and physicochemical properties to determine their degree of unsaturation and oxidative stability during UV irradiation.

2. Materials and methods

2.1. Materials

Brown flax seeds come from the Khemisset region (33° 49′ 00″ Nord, 6° 04′ 00″ Ouest) of Morocco. The flax seeds are harvested towards the end of May 2022, then separated from the packaged capsules and sent to the laboratory, where they are stored at 4 °C, for subsequent cold-pressing.

2.2. Chemicals and reagents

All reagents used in this study, including Folin-Ciocalteu reagent, phenolphthalein ($C_{20}H_{14}O_4$), sodium thiosulfate ($Na_2S_2O_3$), and potassium hydroxide (KOH), as well as solvents like cyclohexane, ethanol, chloroform, and acetic acid, were of analytical grade and sourced from local suppliers.

2.3. Irradiation of flaxseed oil

A 500 g mass of brown flaxseed, with a moisture content of 4.03 %, was cold-pressed, the resulting oil was collected in a 500 mL beaker placed in a sealed light-proof chamber, kept in suspension by magnetic stirring, and placed 6 cm above an LED matrix Foton UF-LED with a wavelength of 310 nm for durations (1 h - 8 h) at 25 °C. Samples of 35 mL were taken and immediately sent for analysis (Lashko, Chausovsky, Derevianko, & Brazhko, 2019).

2.4. Analysis of chemical properties

2.4.1. Fatty acid composition

Fatty acid methyl esters (FAME) were prepared and analyzed by flame ionization coupled to Varian CP-3800 gas chromatography (GC) fitted with a CP-Wax 52 CB column in accordance with the norm (ISO, P., 2015), the carrier gas is helium, the data are processed with Varian Star Workstation v 6.30 and expressed as relative percentage of each fatty acid, following our previously published methods (Belhoussaine et al., 2024).

$\sum$SFA = [Palmitic acid] + [Stearic acid].
$\sum$MUFA = [Oleic acid] + [minors monounsaturated fatty acids].
$\sum$PUFA = [Linoleic acid] + [α-linolenic acid]

2.4.2. Phytosterols composition

Phytosterols composition was defined according to the method of "ISO 6799" (ISO, E., 2014), using our previously published methods (El Bernoussi et al., 2024).

Analysis of the chemical composition of sterols involves converting sterols into trimethylsilyl ethers using the following protocol, 2.5 g of oil was placed in a 250 mL flask mixed with 25 mL of potassium hydroxide solution (1 N ethanol) and heated under reflux for 30 mins until the solution became clear. 25 mL of distilled water was then added to stop the reaction. The unsaponifiable matter was extracted using 75 mL of hexane. The organic phase was washed with 15 mL of a mixture (water/ethanol 95°) (90/10) in a separating funnel, and the hexane phase was recovered in a 100 mL flask. After evaporation of the solvent using a rotary evaporator, the unsaponifiable matter was recovered, and diluted with 300 µL of hexane, and filtered. The unsaponifiable matter was separated by preparative silica gel TLC. The mobile phase is: 80 mL hexane +20 mL ethyl acetate. TLC revelation was carried out using fluorescein + alcohol (0.5 fluorescein in 1 L ethanol), after which the sterol band was scraped off and placed in a flask containing 10 mL chloroform. After evaporation of the solvent, the sterols were converted to silyl derivatives (TMS) using a mixture of pyridine, hexamethyldisilizane (HMDS), and trimethylchlorosilane (TMCS), (9/1/1), (*v*/v/v). The pyridine was evaporated to dryness and the silyl derivatives were diluted with 60 mL of heptane. The trimethylsilylation of the crude sterol fraction is followed by the analysis carried out through a Varian 3800 instrument equipped with a VF-1 ms column (30 m length, 0.25 mm i.d.), and as carrier gas there is helium (flow rate 1.6 mL/mins) at a constant temperature of 270 °C of the column and those of the detector and the injector is 300 °C, the amount injected was 1 µL for each analysis, adding the internal standard remains 5α-cholestane. The results of the sterol analysis are expressed in (mg/kg).

2.4.3. Tocopherols composition

The tocopherol composition was determined according to the "ISO 9936" method (ISO, I. J. I. O. f. S. G., Switzerland, 2016) by means of high performance liquid chromatography (HPLC) using a solution of 250 mg of oil in 25 mL of n-heptane, *via* Shimadzu CR8A instruments connected to a C18-Varian column, and then Detection is carried out using a fluorescence detector (excitation wavelength 290 nm − emission wavelength 330 nm) on a silica column (25 cm × 4 mm). The mixture of isooctane/isopropanol (99:1) (*V*/V) occupies the eluent and α-tocopherol acts as the external standard, using our previously published methods (Belhoussaine et al., 2024).

2.4.4. Total phenolic contents (TPC)

Polyphenol content is determined based on the Folin-Cio-calteu method referring to gallic acid. This method was performed

accordingly to (Hrncirik & Fritsche, 2004) with minor modifications.

2.5. Physicochemical properties

The oxidative stability of cold-pressed oils throughout their exposure to UV light was determined using the American Oil Chemists' Society (AOCS, O. S. I., 1998), by the free fatty acids (FFA) (Animal, 2009), peroxide value (PV) (ISO, 2012), iodine value (IV) (ISO, 1996), specific extinction coefficients (K232 and K270) (ISO, I. S. O., 2011), and colour value (CV) (15305, I, 1998), also determination of chlorophylls and carotenoids pigments after (1–8 h) of irradiation (Gharby et al., 2018).

FFA (% of oleic acid); Peroxide value (meq O_2/kg oil); Iodine value (mg I_2/ Kg oil); specific extinction coefficients (K232 and K270); carotenoids and chlorophylls were expressed as in our previously published methods (Belhoussaine et al., 2024). The colour index is determined by the spectrophotometric absorbance at 420 nm of a 2.5 % *w/v* oil solution in isooctane using a Shimadzu spectrophotometer.

2.6. Nutritional indexes

Nutritional properties were evaluated using different indexes: the atherogenic index (AI), the thrombogenic index (TI), oxidazability value (Cox), also the oxidative susceptibility (OS), and the hypocholesterolemic: hypercholesterolemic ratio (HH).

The formulas for calculating (AI) and (TI) appear to be (Hashempour-Baltork, Torbati, Azadmard-Damirchi, & Savage, 2018):

$$AI = \left[((4 \times C14:0) + C16:0 + C18:0) \Big/ \left(\sum MUFA + \sum \omega_6 \times PUFA + \sum \omega 3 \times PUFA\right)\right] \quad (1)$$

$$TI = [(C14:0 + C16:0 + C18:0)]/[(0.5 \times MUFA) + (0.5 \times \omega_6 \times PUFA) + (3 \times \omega_3 \times PUFA) + (\omega_3/\omega_6 \times PUFA)] \quad (2)$$

and the Cox's equation is (Fatemi & Hammond, 1980):

$$Cox[C181103C182216C183]/100 \quad (3)$$

Oxidative susceptibility (OS) was calculated using the formula according to (Cecchi, Passamonti, Alfei, & Cecchi, 2011):

$$OS = [MUFA + (45 \times C18:2) + (100 \times C18:3)] \quad (4)$$

The degree of unsaturation of lipids was analyzed in term of oleyl- (ODR), and linoleyl- (LDR) desaturation ratios:

$$ODR = [(C18:2 + C18:3)/(C18:1 + C18:2 + C18:3)] \times 100 \quad (5)$$

$$LDR = [(C18:3)/(C18:2 + C18:3)] \times 100 \quad (6)$$

2.7. Statistical analysis

Each variable was studied in triplicate, and results were expressed as the mean of the three independent one-sample measurements ($n = 3$) ± SD (n = 3). Statistical analyses were performed using GraphPad Prism software version 10.1.2 (324). An analysis of variance (ANOVA) was performed using a two-way followed by Tukey's significant difference test ($p < 0.05$). An analysis of correlation was performed by Pearson's test.

3. Results and discussions

3.1. Extraction yield of L. usitatissimum oil

Extraction of brown flaxseed by cold pressing revealed a high oil yield of 36.18 %, with similar contents ranging from 35 to 44 %, 32.56 and 39.98 % (Klein, Zikeli, Claupein, & Gruber, 2017; Yaqoob, Bhatti, Anwar, Mushtaq, & Artz, 2016), which was close to the oil yield reported respectively 26.0–27.4 % (Suri, Singh, Kaur, & Yadav, 2023; Zanqui et al., 2015), also in comparison with other vegetable oils such as *Helianthus annuus* L. (21.4 %) (Nadeem et al., 2015), *Carthamus tinctorius* L. (38 and 48 %) (Aydeniz, Güneşer, & Yılmaz, 2014), *Glycine* max *merilli* (18 and 20 %) (Moses, 2014), and *Arachis hypogea* L. (41.5 %) (Abdelghany et al., 2022).

3.2. Fatty acids composition changes in irradiated oils

Table 1 shows the changes in fatty acid composition under UV irradiation. In all the oils tested, the unsaturated fatty acid content tended to increase with increasing UV exposure time, with remarkable changes observed in oleic, linoleic and α-linolenic acids.

The Flax Council of Canada reports that flaxseed oil typically contains about 9 % saturated fatty acids, 18 % monounsaturated fatty acids, and 73 % polyunsaturated fatty acids (da Silva Moura, da Silva, & Braga, 2023). In comparison, the control flaxseed oil has a fatty acid composition of 10.90 % saturated fatty acids (SFA), 22.03 % monounsaturated fatty acids (MUFA), and 66.721 % polyunsaturated fatty acids (PUFA). It is particularly rich in α-linolenic acid (51.83 %), oleic acid (21.51 %), and linoleic acid (14.88 %), with lower concentrations of palmitic acid and stearic acid, thereby meeting Canadian Flax standards (Canada & o., 2009; Morris, 2007). In the flaxseed oil bibliography, 30 samples of cold-pressed flaxseed oil were analyzed and identified by maximum and minimum values of stearic fatty acid 2.55 % and 4.72 %, palmitic fatty acid 4.83 % and 6.23 %, linoleic fatty acid 11.72 % and 21.87 %, oleic fatty acid 15.16 % and 26.08 %, and linolenic fatty acid 48.68 % and 62.76 % (Kouamé et al., 2021; Mikołajczak & Tańska, 2022; Pointner et al., 2024). The α-linolenic acid content in the oil increased gradually after the first hour of UV exposure, from 51.83 % in the control oil to 52.13 %, and rose significantly to 55.49 % after 8 h. In contrast, other fatty acids did not exhibit significant changes, resulting in an overall increase in the percentage of polyunsaturated fatty acids (PUFA).

On the other hand, a decrease in percentages of fatty acids in irradiated flaxseed oil, perilla oil and green nut oil between 5 h and 15 h is considered, such that for flaxseed oil the concentration of C18:3 initially decreases from 395.7 mg/g oil to 349.3 mg/g oil after 5 h irradiation and down to 333.3 mg/g oil after 15 h. Similarly, C18:2 acid initially decreased from 101.2 to 87.4 and 63.8 during 5 h and 15 h UV exposure respectively, and the same applies to the other fatty acids in the study (Takeyama, Fukushima, & Research, 2013), in a similar manner, sesame oil irradiated for 12 days exhibited a gradual decrease in fatty acid content (Al-Bachir, Koudsi, & International, 2021). Other studies have attempted to expose flaxseed oil to different doses of irradiation, and the results obtained show no significant difference between control and irradiated oils respectively total SFA varies from 11.24 to 11.09 %, total PUFA varies from 68.04 to 67.96 % (Yalcin et al., 2011).

3.3. Nutritional indexes changes in irradiated oils

The nutritional quality of oils has been assessed by nutritional

Table 1
Fatty acids composition during UV irradiation.

		Irradiation time (h)					
Fatty acid (%)	Control	1H	2H	4H	6H	8H	
Palmitic acid	C16.0	5.59 ± 0.02 [a]	5.62 ± 0.03 [b]	5.63 ± 0.01 [b]	5.67 ± 0.0 [c]	5.71 ± 0.03 [d]	5.92 ± 0.09 [e]
Stearic acid	C18.0	4.78 ± 0.01 [a]	4.83 ± 0.06 [b]	4.95 ± 0.09 [c]	4.97 ± 0.02 [d]	5.11 ± 0.13 [e]	5.22 ± 0.01 [f]
Oleic acid	C18.1	21.51 ± 0.01 [a]	21.53 ± 0.02 [a]	21.53 ± 0.02 [a]	21.65 ± 0.05[b]	21.71 ± 0.05[c]	22.00 ± 0.03[d]
Linoleic acid	C18.2	14.88 ± 0.02[a]	14.87 ± 0.02 [a]	14.95 ± 0.04[b]	14.95 ± 0.02[b]	15.14 ± 0.04[c]	15.87 ± 0.07[c]
Linolenic acid	C18.3	51.83 ± 0.02[a]	52.14 ± 0.02 [b]	52.14 ± 0.02[b]	52.17 ± 0.05[c]	52.31 ± 0.06[d]	55.49 ± 0.04[e]
Arachidic acid	C20.0	0.44 ± 0.01 [a]	0.52 ± 0.01 [b]	0.42 ± 0.01 [c]	0.54 ± 0.0 [d]	0.57 ± 0.01 [e]	0.60 ± 0.09 [f]
∑SFA		10.90 ± 0.02[a]	10.97 ± 0.07 [a]	11.10 ± 0.00[b]	11.19 ± 0.02[c]	11.40 ± 0.13[d]	11.75 ± 0.06[e]
∑MUFA		22,03 ± 0.014[a]	22,05 ± 0.01 [a]	22,32 ± 0.01[b]	22,48 ± 0.04[c]	22,61 ± 0.08[d]	23,23 ± 0.02[e]
∑PUFA		66.72 ± 0.04[a]	67.00 ± 0.02 [b]	67.06 ± 0.04 [b]	67.12 ± 0.02[c]	67.46 ± 0.11[d]	71.36 ± 0.07[e]

Each replicate is represented as the mean of three ($n = 3$ e ± SEM). Values in the same row with different superscript letters are significantly different ($p < 0.05$).
∑SFA; satured fatty acids.;
∑MUFA; monounsaturated fatty acids;
∑PUFA; polyunsaturated fatty acids;

Table 2
Nutritional indexes of oil during UV irradiation.

	Irradiation time (h)					
	Control	1H	2H	4H	6H	8H
AI	0.002 ± 0.00 [a]	0.002 ± 0.00 [a]	0.002 ± 0.00 [a]	0.002 ± 0.00 [a]	0.002 ± 0.00 [a]	0.002 ± 0.00 [a]
TI	0.001 ± 0.00 [a]	0.001 ± 0.00 [a]	0.001 ± 0.00 [a]	0.001 ± 0.00 [a]	0.001 ± 0.00 [a]	0.001 ± 0.00 [a]
Cox	12.94 ± 5.35[a]	13.00 ± 2.22[b]	13.01 ± 5.19[c]	13.02 ± 7.94[d]	13.07 ± 1.94[e]	13.84 ± 6.74[f]
OS	5875.49 ± 0.30[a]	5904.57 ±1.06[b]	5908.49 ±2.16 [c]	5912.49 ±0.31[d]	5935.65 ±0.86[e]	6286.91 ±3.07[f]
ODR	75.62 ± 0.01[a]	75.67 ± 0.02 [b]	75.70 ± 0.012[c]	75.61 ± 0.05[d]	75.64 ± 0.02 [e]	76.43 ± 0.04 [f]
LDR	77.69 ± 0.02[a]	77.79 ± 0.02 [b]	77.75 ± 0.05 [c]	77.72 ± 0.04[d]	77.54 ± 0.02 [e]	77.75 ± 0.00 [f]
PUFAs/SFAs	6.11 ± 0.00 [a]	6.10 ± 0.03 [b]	6.03 ± 0.00 [c]	6.05 ± 0.01 [d]	5.91 ± 0.07 [e]	6.07 ± 0.02 [f]
ω_3/ω_6	3.48[a]	3.50 [b]	3.54 [c]	3.48 [d]	3.45 [d]	3.49 [d]

Each replicate is represented as the mean of three (n = 3 e ± SEM). Values in the same row with different superscript letters are significantly different (p < 0.05).

indices such as AI, TI, HH, Cox, OS, these indices evaluate the nutritional quality of oils according to their fatty acid composition and are presented in Table 2.

AI and TI are indicators of cardiovascular disease risk, and it is recommended to keep these indices at low levels as part of a healthy diet, AI value should be less than 1.0 and TI value should be less than 0.5 (Hashempour-Baltork et al., 2018). In our study AI and TI seem stable in irradiated oils for 8 h, although for the control oil, such as AI = 0.002 < 0.5 and TI = 0.001 < 1. 0, similar to sweet cherry, pomegranate, pumpkin and sunflower oils with AI values (0.15, 0.42, 0.34 and 0.05 < 0.5) and TI values (0.30, 0.75, 0.65 and 0.16 < 1.0) respectively (Machate et al., 2022; Siano, Straccia, Paolucci, Fasulo, Boscaino, Volpe, et al., 2016) indicate better nutritional quality due to the cardioprotective effect of the high PUFA content (Bielecka, Ziajka, Staniewski, & Nowak, 2023; da Silva Moura et al., 2023).

The oxidizability (Cox) and oxidative susceptibility (OS) indices determine the oxidative stability of the oil, so the OS index and the Cox index should be as low as possible to indicate that the fatty acids are less likely to oxidize and therefore the oil is stable against oxidation. Cox and OS values increase in parallel with the increase in unsaturated fatty acids, and the longer the exposure, the more important the UFAs become in the oil, as shown by the difference between the control oil and the oil after 8 h of UV exposure rising from 12.944 to 13.841, indicating low oxidative stability of the oil against UV radiation (Hassanein et al., 2022), In contrast to the oxidizability values of 1.56 for palm oil, 6.53 for maize oil, and 4.82 for rapeseed oil, which indicate greater stability, flaxseed oil appears to be less stable (Athanasiadis, Kalompatsios, Mantiniotou, & Lalas, 2024).

The significant variation in the percentages of oleic and linoleic acids (Table 1) also appears for the LDR and ODR ratios, with all oils containing high levels of both acids and showing no difference in the linoleic (LDR) and oleic (ODR) desaturation ratios during the hours of exposure. The relatively high ODR (75–76) and LDR (77) values account for the increase in the high C18:3 content, as recently demonstrated by the ODR (73–82) and LDR (77–80) (Mengistu, Abu, & Amsalu, 2022) ratios, while sesame oil shows low ODR and LDR ratios (Mondal, Bhat, & Srivastava, 2010), overall, low ratios were observed for sesame oils (ODR = 0.5 and LDR = 0.01) and false flaxseed (Camelina) oils (ODR = 0.74 and 0.82; LDR = 0.56 and 0.66) (Blume & Rakhmetov, 2017).

The ratios ($\omega 3/\omega 6$) and (U/S) do not show changes during the hours of exposure, such that the ratio (U/S) varies between 6.11 and 5.91, and ($\omega 3/\omega 6$) varies from 3.48 to 3.50. In human nutrition, the ($\omega 3/\omega 6$) ratio should be less than 0.02 (Jankowska, Zakęś, Żmijewski, & Szczepkowski, 2010), and a low (UFAs/SFA) ratio indicates better stability of blended oils (Karupaiah & Sundram, 2013), and thus an increase in postprandial HDLC (Bhatnagar, Prasanth Kumar, Hemavathy, Gopala Krishna, & o. t. A. O. C. S., 2009).

3.4. Phytosterols contents changes in irradiated oils

Six phytosterols were identified by GC analysis of the oil samples: 1, β-sitosterol; 2, campesterol; 3, stigmasterol; 4, Δ-5-avenasterol; 5, cholesterol; 6, Δ-7-Avenosterol.

Although the phytosterols in flaxseed were primarily composed of β-sitosterol and campesterol, the total phytosterol content varied from 4344.1 mg/kg in the control oil to 3786.53 mg/kg after eight hours of UV exposure. These results are consistent with those found in similar oils from other sources, where total contents were reported as follows: 3308.9–3494.1-4344.1 mg/kg (Matthäus, Özcan, & Engineering, 2017), and 2888.1–3277.6-3475.6 mg/kg (Zeng et al., 2022a).

β-Sitosterol remained the predominant sterol in all flaxseed oil samples (Table 3), ranging from 1911.24 mg/kg in the control to 1815.65 mg/kg after 8 h of UV exposure. Campesterol levels varied from 1147.25 mg/kg (control) to 1047.82 mg/kg (after 8 h), stigmasterol levels ranged from 454.05 mg/kg (control) to 400.02 mg/kg (after 8 h), while, similarly, Δ-5-avenasterol levels decreased from 438.17 mg/kg (control) to 374.56 mg/kg (after 8 h). The observed differences were statistically significant ($p < 0.05$). The phytosterol content in irradiated

Table 3
Phytosterols content during UV irradiation.

	Irradiation time (h)						
Phytosterols (mg/Kg)	Control	1H	2H	4H	6H	8H	Loss of phytosterols (%)
Cholesterol	37.40 ± 0.01[a]	34.71 ± 0.02[b]	31.90 ± 0.09 [c]	30.66 ± 0.06[d]	29.81 ± 0.04 [e]	29.67 ± 0.03 [f]	22.66 ± 0.07 %
Campesterol	1147.25 ± 0.02[a]	1088.06 ± 0.01[b]	1082.35 ± 0.01[c]	1076.41 ± 0.04[d]	1053.22 ± 0.01[e]	1047.82 ± 0.0 [f]	8.66 ± 0.04 %
Stigmasterol	454.05 ± 0.01[a]	451.19 ± 0.05[b]	433.11 ± 0.03 [c]	418.30 ± 0.02[d]	403.16 ± 0.06 [e]	400.02 ± 0.01 [f]	11.89 ± 0.02 %
β-Sitosterol	1911.24 ± 0.01[a]	1902.51 ± 0.02[b]	1861.43 ± 0.01 [c]	1847.03 ± 0.02[d]	1823.17 ± 0.07[e]	1815.65 ± 0.04[f]	5.00 ± 0.05 %
Δ-5-Avenosterol	438.17 ± 0.08[a]	431.32 ± 0.05[b]	429.93 ± 0.01 [c]	414.18 ± 0.01[d]	387.19 ± 0.02 [e]	374.56 ± 0.01 [f]	14.51 ± 0.04 %
Δ-7-Avenosterol	27.11 ± 0.03[a]	25.94 ± 0.02[b]	23.73 ± 0.01 [c]	20.45 ± 0.01[d]	19.05 ± 0.04 [e]	18.63 ± 0.08 [f]	31.28 ± 0.06 %
Total	4344.70 ± 0.01[a]	4073,08 ± 0.02[b]	3962.83 ± 0.01[c]	3873,97 ± 0.07[d]	3795.86 ± 0.05[e]	3786.53 ± 0.02[f]	12.84 ± 0.09 %

Each replicate is represented as the mean of three (n = 3 e ± SEM). Values in the same row with different superscript letters are significantly different ($p < 0.05$).

Table 4
Tocopherols content during UV irradiation.

	Irradiation time (h)						
Tocopherols (mg/Kg)	Control	1H	2H	4H	6H	8H	Loss of tocopherols (%)
α-tocopherols	5,03 ± 0.03[a]	2,42 ± 0.01 [b]	2,02 ± 0.1 [c]	1,93 ± 0.08[d]	1,91 ± 0.04 [e]	1,88 ± 0.06 [f]	62.62 ± 0.11
γ-tocopherols	647,51 ± 0.21[a]	645,14 ± 0.11[b]	610,07 ± 0.03[c]	555,12 ± 0.05[d]	453,41 ± 0.01[e]	317,47 ± 3.14[f]	50.97 ± 0.08
δ-tocopherols	12,93 ± 0.06 [a]	10,04 ± 0.09[b]	9,78 ± 0.2 [c]	8,5 ± 0.03 [d]	7,86 ± 0.06 [e]	5,38 ± 0.043 [f]	58.39 ± 0.09
Total	668,54 ± 0.87[a]	659,91 ± 0.42[b]	628,14 ± 0.71[c]	567,01 ± 0.57[d]	465,33 ± 0.53[e]	323,12 ± 7.36[f]	51.66 ± 0.81

Each replicate is represented as the mean of three (n = 3 e ± SEM). Values in the same row with different superscript letters are significantly different ($p < 0.05$).

flaxseed oils remains reliable and aligns with the percentage reported in previous studies, therefore, it preserves a beneficial phytosterol profile (Gandova, Teneva, Petkova, Iliev, & Stoyanova, 2023; Matthäus et al., 2017; Zeng et al., 2022b).

3.5. Tocopherol contents changes in irradiated oils

Table 4 presents the tocopherol content of the sampled oils in comparison to the control flaxseed oil during UV exposure. Specifically, *γ*-tocopherols are the most abundant at 647.514 mg/kg, followed by *δ*-tocopherols at 12.938 mg/kg, *α*-tocopherols at 5.039 mg/kg, resulting in a total tocopherol content of 668.548 mg/kg. Similar to other research, the total tocopherol content is approximately 614.9 mg/kg, with *γ*-tocopherols at 601.0 ± 0.32 mg/kg, *α*-tocopherol at 8.6 ± 0.06 mg/kg, and *δ*-tocopherols at 5.4 ± 0.01 mg/kg (da Silva Moura, da Silva, & Braga, 2023), consistent with this, the results reported (Shadyro et al., 2020) indicated *γ*-tocopherol levels ranging from 494 to 586 mg/kg, *δ*-tocopherol levels from 23 to 26 mg/kg, *α* -tocopherol levels from 14 to 17 mg/kg, and total tocopherol content ranging from 537 to 623 mg/kg, while lower levels of *γ*-tocopherol were recently found, at 328 mg/Kg (Pointner et al., 2024).

A decrease in total tocopherol content is clearly observed between the sampled oils and the control oil. Initially, a total of 668.548 mg/Kg, which degrades with increasing UV exposure, reaching 323.124 mg/Kg at the end of the experiment. *γ*-tocopherols, the majority isomer, showed a pre-exposure content of 647.514 mg/Kg, which declined significantly after 2 h under UV radiation to 610.072 mg/Kg, then 555.123 at (4 h), then 453.415 at (6 h), and finally 317.472 mg/Kg. The oil's *δ*-tocopherol content in turn is sharply reduced throughout the duration of UV exposure, arriving proximately at 50 % (7.862 mg/Kg) of its loss after 6 h and ending at a concentration of 5.386 mg/Kg. The observed degradation of tocopherols is due to their high sensitivity to light, as they tend to protect unsaturated oils and fatty acids during the oxidation process (Musakhanian, Rodier, & Dave, 2022). In terms of irradiation doses, exposure of the oil to various gamma doses shows reductions in *γ*-tocopherol content of 17 % at 5 kiloGray (kGy), and 15 % at 3 kiloGray (KGy) respectively (Beheshti Moghadam, Rezaei, Behgar, Kermanshahi, & Chemistry, 2019; Lakritz et al., 1995).

3.6. Phenolic, chlorophylls and carotenoids contents in irradiated oils

Table 5 shows the progression of carotenoid and chlorophyll pigment concentrations, as well as phenolic compound content in oils during the hours of UV exposure. The loss of carotenoid concentration and the increase in chlorophyll concentration demonstrate the influence of UV radiation on oil quality, while the decrease in phenolic compounds in the oils sampled demonstrates the low oxidative stability of flaxseed oil against UV radiation.

The control flaxseed oil showed an average carotenoid content of 0.12 mg/kg and after 8 h of UV exposure, the oil saw a 58.33 % decrease in its carotenoid content. Similar to the value of 0.16 mg/kg reported by (da Silva Moura, da Silva, & Braga, 2023), higher levels were observed, ranging from 2.89 to 43.6 mg/kg, and reaching up to 57.3 mg/kg (Mikołajczak & Tańska, 2022; Mohanan, Nickerson, & Ghosh, 2018; Suri, Singh, Kaur, Yadav, & Singh, 2020), However, this content is lower than that in some almond oil varieties, which is 0.58 mg/kg, and higher than in others, which is 0.07 mg/kg (El Bernoussi et al., 2024).

In the presence of light and atmospheric oxygen, the decomposition of chlorophylls produces pheophytins and pheophorbides, which act as sensitizers, accelerating oil oxidation by forming 1 O_2 (Endo, Usuki, Kaneda, & o. t. A. O. C. S., 1984; Rahmani, Csallany, & o. t. A. O. C. S., 1998), whereas in the dark, they are characterized by antioxidant

Table 5
Phenolic, Carotenoids and Chlorophylls contents during UV irradiation. PERTE.

	Irradiation time (h)						
	Control	1H	2H	4H	6H	8H	Loss of contents (%)
Carotenoids (mg/Kg)	0.12 ± 0.09 [a]	0.11 ± 0.08 [a]	0.11 ± 0.07 [a]	0.09 ± 0.01 [a]	0.08 ± 0.04 [b]	0.05 ± 0.04 [c]	58.33 ± 0.07
Chlorophylls (mg/Kg)	0.26 ± 0.01 [a]	0.49 ± 0.03 [b]	0.69 ± 0.02 [c]	0.71 ± 0.05 [d]	0.74 ± 0.02 [e]	0.79 ± 0.03 [f]	–
Total polyphenols (mGAE/g oil)	5.85 ± 0.09 [a]	4.48 ± 0.09[b]	3.24 ± 0.19 [c]	2.97 ± 0.67 [d]	1.32 ± 0.09 [e]	0.92 ± 0.19 [f]	84.27 ± 0.21

Each replicate is represented as the mean of three (n = 3 e ± SEM). Values in the same row with different superscript letters are significantly different ($p < 0.05$).

properties, probably due to the transfer of hydrogen to free radicals (Endo, Usuki, Kaneda, & o. t. A. O. C. S., 1985; Gutiérrez-Rosales et al., 1992). The chlorophyll content recorded for the control oil is 0.26 mg/kg in its initial state, which is less than the 0.61 mg/kg previously determined (Pointner et al., 2024), and it more than doubled after 2 h of exposure, reaching 0.79 mg/kg in the final sampling. Despite these increases, all samples remained within the range of recent studies, which reported 2.34 mg/kg and 5.76 mg/kg (Choo, Birch, & Dufour, 2007; Herchi et al., 2014).

Phenolic compounds are natural secondary metabolites that present many biological effects, where the antioxidant capacity is the most important characteristic mainly for its beneficial impact on health (Rahman et al., 2021). The polyphenol content of the pure control oil is 5.850 mg GAE/ g oil extract, which is considerably higher than the 2.85 mg GAE/100 g oil extract, the 0.27 mg GAE/100 g oil extract and 15.4 mg GAE/kg reported (Fruehwirth et al., 2020; Kostadinovik & Mitrev, 2013; Pointner et al., 2024) compared to *Cucurbita pepo* oil has a similar polyphenol content of 5.37/g oil extract, while Argan oil has a significantly higher content of 23.44 mg GAE/g oil extract (Boujemaa et al., 2024; El Idrissi et al., 2023). Phenolic compounds in the oil began to decline significantly from the second hour of UV exposure, with a content of 3.246 mg GAE/100 g oil. Losses of 4.522 mg GAE/100 g oil were recorded after 6 h, and 4.93 mg GAE/100 g oil after 8 h resulting in a loss of polyphenols of up to 84.27 %.

3.7. Changes in physicochemical properties of irradiated oils

To assess the main physicochemical parameters of the oil, 6 descriptors were selected, at the start and end of UV exposure: acidity, peroxide value, iodine value, conjugated dienes and trienes (K232-K270), and colour value (Table 6).

3.7.1. Free fatty acid

During the eight hours the oils were exposed to UV light, the percentage of free fatty acids in the oils increased correlatively with time. The control oil shows 0.18 % of fatty acids a low percentage compared to 0.69 % and 0.595 % of recent studies (Mikołajczak, Pilarski, Gęsiński, & Tańska, 2023; Zeng et al., 2022b). A significant 1.3-fold increase in the percentage of free fatty acids, from 0.18 % to 0.24 %, and significant stability between 2 h and 4 h of exposure. Moreover, studies show a 2.8-fold increase in the acidity of an oil irradiated for 5 weeks, as well as the acid value of sunflower oil irradiated for 4 weeks rising from 1.29 mg KOH/g oil to 2.3 mg KOH/g oil (Lashko et al., 2019).

3.7.2. Peroxide value

The control flaxseed oil has a peroxide value of 2.5 meq O_2/kg, which aligns with the range (2.24 meq O_2/kg – 4.60 meq O_2/kg reported in the literature (Suri et al., 2023; Symoniuk, Ratusz, & Krygier, 2017). This range spans from 1.80 meq O_2/kg (Choo et al., 2007) to 3.94 meq O_2/kg (Pan et al., 2020), extending to 5.42 meq O_2/kg (Khattab & Zeitoun, 2013) and even up to 17.5 meq O_2/kg in some cases (Pointner et al., 2024).

Exposure of oil to UV for 1 h reveals a twofold increase in the index at 5 meq O_2/kg, which remains stable over the following two hours of exposure. After 4 h, the index rose to 6.25 meq O_2/kg, then to 10 meq O_2/kg and finally to 12.5 meq O_2/kg after 8 h, Therefore, PV increased most significantly 5.55 times, from 2.25 meq O_2/kg to 12.5 meq O_2/kg, compared to the initial high peroxide value (PV) of irradiated flaxseed oil, which is 25 meq O_2/kg, it then increased to 174 meq O_2/kg after 5 h, and to 325 meq O_2/kg after 10 h, and to 350 meq O_2/kg after 15 h of UV exposure, perilla oil, on the other hand, has a lower PV than flax, remaining at the limit of 150 meq O_2/kg after 10 h of irradiation, whereas green nuts oil does not exceed 150 meq O_2/kg after 15 h of irradiation (Takeyama et al., 2013), on the other hand, sunflower oil exposed to UV for 4 h does not show a wide variation in peroxide value ranging between 0.50 and 0.60 mmol ½ O/kg (Lashko et al., 2019).

During the first 2 h of the experiment under UV light, the PV remained virtually unchanged, thus confirming a short-wave (UV) radiation effect on oxidation processes, compared to that of long-wave radiation, an effect proven by recent studies (Lashko et al., 2019).

3.7.3. Iodine value

The iodine index measures the average degree of unsaturation of fats and oils and is used as a predictor of lipid oxidation, it is represented by the α-linolenic acid and linoleic acid contents of oils (Shahidi, Zhong, & Products, 2005). The high IV values of all samples prove the presence of unsaturated bonds, and they certainly contain more unsaturated fatty acids (Charef, Yousfi, Saidi, & Stocker, 2008).

UV treatment of the oil revealed an increase in the iodine value from 188.07 g I_2/100g to 200.35 g I_2/100g, 1.06 times higher during 8 h. IV stability is marked between the control and the first oil sample (188 g I_2/100g), as well as between samples taken after 2 h and 4 h (189 g I_2/100g), and also between the samples taken after 2 h and 4 h (189 g I_2/100g). Other studies also found high values 97.24 g I_2/100g, 118.21 g I_2/100 g (Adam Omer Ishag et al., 2020; Jang et al., 2020).

3.7.4. Specific extinction coefficients (K232 and K270)

Conjugated dienes (CDs) appear only with an absorption peak at around 232 nm (Ramadan & Wahdan, 2012), which are primary oxidation products resulting from oxidation of PUFAs (Weber, Bochi, Ribeiro, Victório, & Emanuelli, 2008), to which are added conjugated trienes that absorb in the ultraviolet at 270 nm and designate secondary oxidation products (Abdulkarim et al., 2007). A high level of conjugated dienes gives the oil low oxidative stability (Mohdaly, Sarhan, Mahmoud, Ramadan, & Smetanska, 2010).

During irradiation, the production of primary oxidation compounds shows an evolution with a large difference between the control sample and the samples at each instant, as flaxseed oil shows 1.97 before the experiment and it ends with 2.82. A significant difference appears in the oil from the first hour giving an absorbance of 2.04 then going to 2.24 after 4 h, and 2.63 after 6 h. While secondary oxidation products increase slowly during irradiation, ranging from 0.42 to 0.45 after 8 h. Previous results also demonstrate the variation of 232 values in flaxseed oil approximately between 1.7 and 2.8 (Choo et al., 2007; da Silva

Table 6
Physicochemical properties of oil during UV irradiation.

	Exposition time (h)					
	Control	1H	2H	4H	6H	8H
FFA (% oleic)	0.18 ± 0.05^{a}	0.19 ± 0.02^{b}	0.20 ± 0.00^{c}	0.20 ± 0.01^{c}	0.21 ± 0.08^{d}	0.24 ± 0.00^{e}
PV (meqO_2/kg)	2.25 ± 0.35^{a}	5 ± 0.00^{b}	5 ± 0.00^{b}	6.25 ± 1.76^{d}	10 ± 7.07^{d}	12.5 ± 0.00^{e}
IV (g I_2/100g)	188.07 ± 0.22^{a}	188.88 ± 0.17^{b}	189.01 ± 0.31^{b}	189.24 ± 0.10^{d}	190.05 ± 0.45^{e}	200.35 ± 0.27^{f}
K 232	1.98 ± 0.09^{a}	2.04 ± 0.03^{b}	2.13 ± 0.04^{c}	2.24 ± 0.02^{d}	2.59 ± 0.06^{e}	2.82 ± 0.01^{f}
K 270	0.42 ± 0.01^{a}	0.43 ± 0.01^{b}	0.43 ± 0.02^{b}	0.43 ± 0.00^{b}	0.44 ± 0.00^{e}	0.45 ± 0.01^{f}
CV	0.18 ± 0.00^{a}	0.23 ± 0.02^{b}	0.23 ± 0.01^{b}	0.25 ± 0.00^{d}	0.28 ± 0.00^{e}	0.34 ± 0.02^{f}

Each replicate is represented as the mean of three (n = 3 e ± SEM). Values in the same row with different superscript letters are significantly different ($p < 0.05$). Free fatty acid (FFA); peroxide value (PV); iodine value (IV); specific Extinction coefficients (K 232 and K 270); Colour value (CV).

Moura et al., 2023), as well as 270 values between 0.16 and 0.3. A statistically significant increase ($p < 0.05$) in the values of both extinction coefficients was observed during the eight-hour experiments.

3.7.5. Colour value

Oleaginous seeds and fruits contain pigments that give different colours to vegetable oils; the presence of carotenoids is indicated by red and yellow hues, such as red is due to carotene and yellow one due to xanthophylls (Mortensen, Skibsted, Truscott, & Biophysics., 2001).

Colour is an important physical characteristic of lipids. As the UV light treatment progressed, the colour of the lipids gradually changed from yellow to brown between 1 h and 4 h exposure, then to dark brown after 6 h, and finally to intense brown after 8 h exposure. Under these exposure conditions, the colour value shown in Table 6 increased in the oil by a factor of 1.91. The increase in colour value is due to the oxidation of carotenoids by light. When the oil was stored away from light, this parameter did not change (Lashko et al., 2019).

4. Correlation

To explore the relationship between quality and nutritional indices and the fatty acid (C16:0, C18:1, C18:2 and C18:3), phytosterol and tocopherol contents of L. *usitatissimum* oils, the correlation coefficient of these proportions, as in our previous study (El Kourchi et al., 2024a, 2024b), is shown in Fig. 1.

The qualitative properties of the lipid fraction of all oils (PV, IV, K232, K270, A420, Cox, ODR) showed a positive correlation with fatty acid contents (C16:0, C18:1, C18:2 and C18: 3). These indices tell us about the degree of unsaturation by IV similar to the high abundance of C18:3 and the degrees of unsaturation ODR and COX; and the degree of oxidation by PV, K232, K270, ODR are identical to the calculated oxidizability (Cox). Unlike LDR, which shows the degree of linoleyl desaturation and is negatively correlated with FA and K232 and K270, and moderately correlated with C18:3 content showing the low α-linolenic transformation. Chlorophyll pigments also correlated positively with PV, K270 and A420, proving the existence of conjugated dienes and influencing oil colour during irradiation. The colour index declares a strong positive correlation with quality parameters and FA content, while a negative correlation is marked with tocopherols and phytosterols and polyphenol content, the latter playing the role of antioxidants in the oil and interfering with its deterioration, while the transformation of colour to brown is due to fatty acids and chlorophylls which are oil pro-oxidants. Phytosterols, tocopherols and phenolic compounds are strongly positively correlated with carotenoid pigments, and they are oil protectors against degradation; they are widely present in oil samples so they are sufficiently protected against UV irradiation during 8 h.

5. Conclusion

UV irradiation of cold-pressed flaxseed oil revealed significant variations in the phytosterol and tocopherol compound profiles, which showed a significant decrease during 8 h of UV exposure. In addition, a large loss of phenolic and carotenoid compounds was reported after 4 h of irradiation. While the level of polyunsaturated fatty acids remained stable during 6 h of exposure and then increased to 71.36 %, the chlorophyll concentration increased 2-fold after 2 h of irradiation compared to the control oil. These changes were accompanied by an increase in the levels of FFA, PV and conjugated diene as the exposure time was increased. In terms of the nutritional quality of irradiated oils, the AI, TI, Cox, SO, ODR and LDR indices reveal a high nutritional value throughout UV exposure. The correlation test between the various qualitative and quantitative parameters indicate that irradiation can affect the quality of irradiated oils, but they maintain good oxidative stability during 8 h of exposure, due to their high content of γ- and δ-tocopherols, carotenoid pigments, and polyphenols.

Availability of data and materials

not applicable.

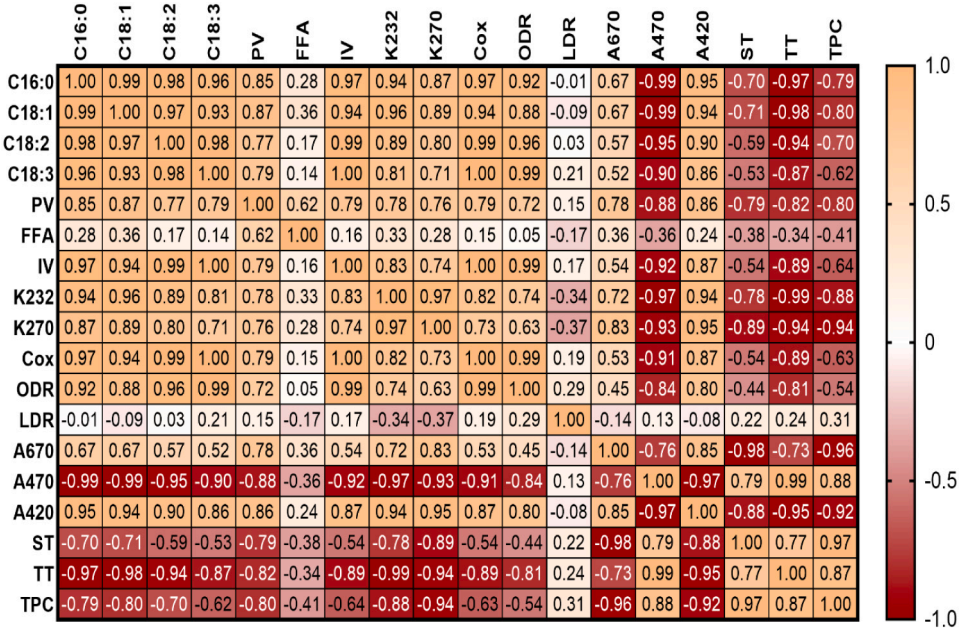

Fig. 1. Nutritional indexes and physicochemical properties of the lipid fraction of L. *usitatissimum* and their chemical compositions are correlated according to Pearson's correlation coefficient.
Free fatty acid (FFA); peroxide value (PV); iodine value (IV); dienes and trines conjugated (K 232 and K 270); colour value (CV); oxidazability value (Cox); linoleyl-desaturation ratios (LDR); oleyl- desaturation ratios(ODR), Chlorophylls and carotenoids contents (A670 and A470); total phytosterols (ST); total tocopherols (TT); Total phenolic compounds (TPC).

CRediT authorship contribution statement

Oumayma Belhoussaine: Writing – original draft, Resources, Investigation, Formal analysis, Data curation. **Chaimae El Kourchi:** Writing – original draft, Software, Resources, Formal analysis. **Amakhmakh Mohammed:** Software, Methodology, Data curation. **Adil EL Yadini:** Writing – review & editing, Visualization, Investigation. **Riaz Ullah:** Writing – review & editing, Investigation, Funding acquisition. **Zafar Iqbal:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis. **Khang Wen Goh:** Writing – review & editing, Validation, Supervision, Funding acquisition. **Monica Gallo:** Writing – review & editing, Visualization, Investigation. **Hicham Harhar:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Abdelhakim Bouyahya:** Writing – review & editing, Validation, Formal analysis, Conceptualization. **Mohamed Tabyaoui:** Writing – review & editing, Project administration, Methodology, Conceptualization.

Declaration of competing interest

Authors declare that there is no conflict of interest.

Data availability

Data will be made available on request.

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