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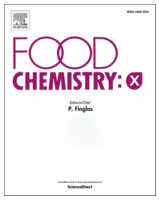

Phytochemical characterization and nutritional value of vegetable oils from ripe berries of *Schinus terebinthifolia* raddi and *Schinus molle* L., through extraction methods

Oumayma Belhoussaine [a], Chaimae El Kourchi [a], Hicham Harhar [a], Hamza El Moudden [b], Adil El Yadini [a], Riaz Ullah [c], Zafar Iqbal [d], Khang Wen Goh [e,f], Bey Hing Goh [g,h], Abdelhakim Bouyahya [i,*], 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] *Higher School of Technology of El Kelaa Des Sraghna, Cadi Ayyad University, El Kelaa Des Sraghna B.P 104, 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] *Sunway Biofunctional Molecules Discovery Centre (SBMDC), School of Medical and Life Sciences, Sunway University, Sunway City, Selangor, Malaysia*
[h] *College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang, China*
[i] *Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, Mohammed V University in Rabat, Morocco*

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ABSTRACT

The aims of this study are the phytochemical exploration and food valorization of *Schinus molle* L. (*S. molle*) and *Schinus terebinthifolia* Raddi (*S. terebinthifolia*) from the Rabat, Morocco. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) were used to analyze the chemical composition of the oils extracted from both species by soxhlet and maceration. Moreover, physicochemical characteristics such as lipid quality indexes such as thrombogenic index (TI), atherogenic index (AI), oxidation susceptibility (OS), and calculated oxidability (Cox) were determined. These characteristics included percentage acidity, peroxide, saponification, iodine, specific extinction values, chlorophyll, and carotenoid pigments. As results, the oil yields varied from 7% (*S. molle*) to 13% (*S. terebinthifolia*). In addition, unsaturated fatty acids represented the major fraction for *S. terebinthifolia* (79%) and *S. molle* (81%). However, *S. terebinthifolia* contains more saturated fatty acids (20%) than *S. molle* (16%) with a predominance of linoleic acid (59.53% and 55%, C18,2), oleic acid (19.29% and 21.69%, C18,1), and palmitic acid (12.56% and 15.48%, C16,0) in *S. molle* and *S. terebinthifolia*, respectively. Moreover, the main sterols are *β*-sitosterol followed by campesterol and then Δ-5-avenasterol, while *β*-sitosterol varies according to the species and the extraction method. Results revealed also that campesterol is influenced by the extraction results in a content of 179.66 mg/kg (soxhlet) and 63.48 mg/kg (maceration) for *S. molle*, while *S. terebinthifolia* yeilds concentrations of 170 mg/kg and 138 mg/kg, then Δ-5-avenasterol, which present with (117 mg/kg and 136 mg/kg), (34 mg/kg and 80 mg/kg) of the total amount of sterols for the oils extracted by soxhlet and maceration, respectively. In addition, there are favorable physicochemical properties for all oils, such as chlorophylls (0.4 to 0.8 mg/kg) and carotenoids (0.7 to 2 mg/kg). However, further investigations are needed to determine other chemical compounds of both extracts as well as to evaluate their biological and health benefits.

* Corresponding author at: Laboratory of Materials, Nanotechnology and Environment, Faculty of Sciences, Mohammed V University in Rabat, Av. Ibn Battouta, B. P1014, Rabat, Morocco
E-mail address: a.bouyahya@um5r.ac.ma (A. Bouyahya).

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1. Introduction

Functional foods consist of biologically and physiologically active compounds (Arshad et al., 2021; Ramakrishna, Sarkar, & Shetty, 2019), which provide health benefits, generally referred to as bioactive compounds BACs (Banwo et al., 2021), are derived from plant, animal or other sources, such as microorganisms (Shetty & Sarkar, 2019). BACs are of great interest due to their diverse biological and functional activities such as antioxidant, anti-inflammatory, anti-diabetic, anti-cancer, anti-viral and anti-tumor activities, protecting the human body from high levels of free radicals and reactive oxygen species (ROS) that can easily react with other molecules, leading to cellular damage (Banwo et al., 2021). A variety of processes are known for vegetable oil extraction, citing chemical, biochemical and mechanical techniques to maximize yields while minimizing alterations to product quality (Gnansounou & Raman, 2017). Studies compare the extraction of oils with and without temperature using n-hexane, and state that temperature increases the diffusivity and solubility of oil, which consequently improves the extraction rate (Premi & Sharma, 2013). And others reported higher oil yields with n-hexane than with chloroform and acetone (Osman, Shigidi, & Elkhaleefa, 2016), hence the choice of the two methods used soxhlet and maceration in order to show the influence of temperature and the duration of contact of the plant material with the solvent.

Schinus terebinthifolia Raddi (Anacardiaceae), indigenous to South and Central America, is also found in subtropical and tropical regions of the USA and Africa (Barbieri et al., 2014). In Brazil, it is also known as the pink pepper or the Brazilian pepper tree. Its ripe berries are red, slightly spicy and sweet and used as a spice in regional culinary dishes (Barkley, 1944; de Lima et al., 2006), which makes the pink pepper berries a sophisticated condiment (Barreira et al., 2023). It is a recommended tree for the reforestation of reservoir margins and the recovery of degraded reserves (de Lima et al., 2006), and as an invasive plant, it covers >30,000 ha of the Florida Everglades (Rodgers, Pernas, & Hill, 2014).

Ethnopharmacological studies showed that *S. terebinthifolia* can be used to treat different diseases such as urinary and respiratory infections, skin wounds and ulcers, tumors, and arthritis (Brandão, Cosenza, Moreira, & Monte-Mor, 2006; Morton, 1978), as well as other illnesses, notably bronchitis, and as agents against fever (febrifuge) and pain (analgesic) (Barbosa, Demuner, Clemente, Paula, & Ismail, 2007; Cavalher-Machado et al., 2008; Gazzaneo, De Lucena, & de Albuquerque, 2005).

In vitro and *in vivo* biological investigations conducted on the berries extracts of *S. terebinthifolia* revealed their biological properties such as anti-diabetic, anti-inflammatory, antioxidant, and antimicrobial effects (Barreira et al., 2023; de Araujo Gomes et al., 2020; de Oliveira et al., 2020; Feuereisen, Barraza, Zimmermann, Schieber, & Schulze-Kaysers, 2017). Moreover, *S. terebinthifolia* exhibited also other medical effects like such as astringent, anti-diarrheal, and diuretic properties (Paiva & Aloufa, 2009).

Schinus molle (Anacardiaceae), or Peruvian pepper, was introduced and naturalized in many countries (Kramer, 1957; Taylor, Suckling, & Rachlinski, 2005) due to its considerable tolerance to drought and heat, its strong potential to compete for nutrients and light, and its rapid growth rate and generous berries production (Demelash, Tigabu, & Odén, 2003; Iponga, Milton, & Richardson, 2008). The pink berries are known as pink peppercorns, and because of their flavor and pungency, they are used as black pepper *(Piper nigrum)* (Giuffrida et al., 2020).

S. molle berries have been used for analgesic, antiseptic, antidepressant, and antibacterial purposes, for respiratory and urinary tract infections as a digestive and diuretic purgative, for toothache, and against rheumatism and menstrual disorders (Barrachina, Bello, Martínez-Cuesta, Primo-Yúfera, & Esplunges, 1997; Duke, 2002; Ferrero, Werdin González, Sánchez Chopa, & Alzogaray, 2006; Machado et al., 2007). In addition, studies have revealed that *S. molle* berries polysaccharides have antioxidant, antigenotoxic, antidiabetic, and anti-hemolytic effects *in vitro*, and anti-inflammatory and antinociceptive properties *in vivo* (Feriani et al., 2020).

Based on the results, the red berries of *S. terebinthifolia* and *S. molle* contain high percentages of ash (14.24% and 17.13%, respectively), protein (10.04% and 12.13%, respectively), fat (25.15% and 22.11%, respectively), and carbohydrate (31.21% and 37.12%, respectively) (de Oliveira et al., 2020; Feriani et al., 2020). Both berries have the same fatty acid profile, characterized by linoleic acid C18:2 (de Oliveira et al., 2020; Ennigrou, Casabianca, Laarif, Hanchi, & Hosni, 2017), and rare studies determine the sterol profile by the dominance of β-sitosterol (Sassi, Elayeb, Karaman, Marzouk, & Mastouri, 2020), while the absence of results on tocopherol content, percentage of free fatty acids in the oil, peroxide value and primary and secondary oxidation products led us to compare our own results with those of *P. lenticus* and *P.atlantica* in the literature.

The aim of this study is to examine the ripe red berries vegetable oils of *S. molle* and *S. terebinthifolia* from Morocco extracted by two methods, identifying their various chemical compositions in fatty acids, phytosterols and tocopherols, as well as to evaluate their physicochemical properties and nutritional values, also to validate the results of previous work on the same species from different origin.

2. Materials and methods

2.1. Plant material

The red berries were harvested around two months after flowering and ripened one month after berries fructification. Samples of ripe red berries of both species are harvested from the beginning of February to the end of March in the region of Rabat on the site (34° 01′53.34″), put in sample bags. One kilogram of ripe, bright red berries hand-picked from four trees located two meters apart, on sandy soil rich in organic matter and in a cool, damp climate. Both species were identified by Prof. El Aboudi Ahmed, a botanist at the Biology Department, Faculty of Sciences, Mohammed V University (Belhoussaine et al., 2022).

On arrival at the laboratory, they are then selected according to their roundness, color (pale red: semi-mature berries) or (red: mature berries), firmness, and absence of wounds), separated from the leaves and stems and kept in their original state without being shelled, then dried in a dry place (20 ± 5 °C) for two weeks and then 40 g of each species ground using an electric grinder to launch the extraction process.

The research team had received full permission to collect the plant from the plantation owner, which is part of the harvesting process for commercial sale purposes. This study complies with local and international biodiversity and species protection regulations. Formal ethical approval is not required because no animal or human subjects are involved.

2.2. Oil extraction

Two extraction methods are present to extract the vegetable oils. Approximately 40 g of the powders were weighed and extracted with the apolar solvent (hexane) in soxhlet equipment (Sox) for 6 h and by maceration (Mac) for 24 h.

After filtration, the solvent was removed using a rotary evaporator under reduced pressure at 50 °C and then the extracts *S. molle* maceration, *S. molle* soxhlet, *S. terebinthifolia* maceration, and *S. terebinthifolia* soxhlet were placed in amber glass vials holding 30 mL of volume closed with plastic threaded lids and stored at 4 °C for one week before moving on to oil analyses.

The extraction yields of all the systems (method/solvent) were determined by the ratio between the mass of extract obtained and the mass of raw material (wet basis), and the results are presented as mean ± standard deviation. Analyses were performed in triplicate. For each species, three extractions were needed to calculate the yield.

2.3. Fatty acid composition

The chemical composition of fatty acids were analysed under conditions described in ISO 12966-4:2015 standard (P. Iso, 2015), the analysis consists of transesterifying glycerides into volatile methyl esters. In order to analyze them by gas chromatography. In a 100 mL flask, a mass of 1 g of the sample is added to a volume of 0.5 mL of methanolic KOH of normality 2, and 10 mL of methanol. Then 1 mL of heptane is added to the reaction mixture, after cooling. The heptane phase containing the methyl esters is transferred to a test tube, and a solution of sodium carbonate, Na_2CO_3 is added. The latter neutralises all the free acids by giving sodium salts with Gharsallaouia release of carbon dioxide. The methyl esters, which are in the organic phase, are removed using a pipette. These are analysed by gas chromatography GC. The HP Hewlett Packard 6890 series GC system is equipped with a splitter injector (T: 240 °C) and an FID (T: 260 °C). The carrier gas is nitrogen (PE: 12.4 bar). The analysis is performed in temperature programming (140 °C to 200 °C with a speed of 10 °C/ min and an isotherm at 200 °C for 60 min) on a capillary column (polyethylene glycol) (30 m length × 0.32 mm, ID, 0.25 µm film thickness). The data are processed with Varian Star Workstation v. 6.30 and expressed as a relative percentage of each fatty acid (Harhar et al., 2019; E. N. Iso).

2.4. Unsaturation levels

The degree of unsaturation of lipids was analysed using double bond index (DBI), and the introduction of double bonds into the hydrocarbon chains of linoleic [C18: 2(n-6)] and α-linolenic [C18:3(n-3)] acids was expressed as oleyl- (ODR), and linoleyl- (LDR) desaturation ratios.

The DBI is expressed following the eq. (1) (Gigon, Matos, Laffray, Zuily-Fodil, & Pham-Thi, 2004):

$$\mathbf{DBI} = [(\mathbf{1} \times \%\mathbf{monoenoic\ acid}) + (\mathbf{2} \times \%\mathbf{dienoic\ acid}) + (\mathbf{3} \times \%\mathbf{trienoic\ acid})]. \quad (1)$$

ODR: oleic desaturation ratio, indicates the desaturation of oleic acid to linoleic acid and LDR: linoleic desaturation ratio, corresponds to that of linoleic acid to linolenic acid in order to evaluate the desaturation of the berries both are calculated according to the eqs. (2) and (3) (Mondal, Bhat, & Srivastava, 2010):

$$\mathbf{ODR} = [(\mathbf{C18:2} + \mathbf{C18:3})/(\mathbf{C18:1} + \mathbf{C18:2} + \mathbf{C18:3})] \times \mathbf{100}. \quad (2)$$

$$\mathbf{LDR} = [(\mathbf{C18:3})/(\mathbf{C18:2} + \mathbf{C18:3})] \times \mathbf{100}. \quad (3)$$

The desaturation rate indicates the quantity of substrate that is fully desaturated from C18:1 to C18:2 and C18:3, which results in a relative measure of the desaturating enzymes for each species (Mondal et al., 2010).

2.5. Sterol composition

The sterol composition was defined according to the ISO 12228-1:2014 method (ISO, E., 2014). 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).

2.6. Tocopherol composition

The tocopherol composition was determined according to the ISO 9936:2016 standard (Animal & Fats, 2011), 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 (Champ sur Marne, France) connected to a C18-Varian column, and then detection is carried out using a fluorescence detector with an excitation wavelength of 290 nm – emission wavelength of 330 nm on a silica column 25 cm length × 4 mm; Varian Inc., Middelburg, The Netherlands.

The mixture of isooctane/isopropanol (99:1) (*v*/v) occupies the eluent, and α-tocopherol acts as the external standard. The results of the tocopherol analysis are expressed in (mg/kg).

2.7. Nutritional indexes

Apart from the ratio (UFA/SFA), the quality assessment of the berries oils is examined by the thrombogenic index (TI), the atherogenic index (AI), the oxidative susceptibility (OS), and the calculated oxydizability (Cox).

Assessing the indexes of atherogenicity (AI) and thrombogenicity (TI) can provide information regarding the different effects that individual FAs could have on human health and, in particular, on the likelihood of an increased incidence of atherosclerosis, the development of blood clots, atheroma, and thrombus formation (Manuela et al., 2011; Ulbricht & Southgate, 1991). AI indicates the relationship between major FAs (lauric, myristic, and palmitic) classified as proatherogenic and antiatherogenic UFAs (Manuela et al., 2011). The incidence of atherogenicity has been associated with various inflammatory and innate immune pathways, and may be used as a preliminary indication of accelerated atherosclerosis (Acay et al., 2014; Kouřimská, Pokhrel, Božik, Tilami, & Horčička, 2021). TI shows a tendency for clots to form in blood vessels. This process is defined as the relationship between prothrombogenic (SFA) and anti-thrombogenic fatty acids (monounsaturated fatty acids (MUFA), n-6, and n-3 PUFA) (Manuela et al., 2011). AI and TI are reliable and widely used indexes for all kinds of fats and oils that can be used to assess the potential effects of FA on cardiovascular disease (Chen & Liu, 2020).

The eq. (4) and (5) were used for calculating (AI) and (TI) respec-

tively according to (Santos et al., 2022; Ulbricht & Southgate, 1991):

$$\mathbf{AI} = [(\mathbf{4} \times \mathbf{C14} : \mathbf{0}) + \mathbf{C16} : \mathbf{0} + \mathbf{C18} : \mathbf{0}] \Big/ \Big[\sum \mathbf{MUFA} + \sum \mathbf{\omega 6} \times \mathbf{PUFA} + \sum \mathbf{\omega 3} \times \mathbf{PUFA}\Big]. \quad (4)$$

$$\mathbf{TI} = [(\mathbf{C14} : \mathbf{0}) + \mathbf{C16} : \mathbf{0} + \mathbf{C18} : \mathbf{0})]/[(\mathbf{0.5} \times \mathbf{MUFA}) + (\mathbf{0.5} \times \mathbf{\omega 6} \times \mathbf{PUFA}) + (\mathbf{3} \times \mathbf{\omega 3} \times \mathbf{PUFA}). + (\mathbf{\omega 3}/\mathbf{\omega 6} \times \mathbf{PUFA})]. \quad (5)$$

and for calculating (Cox), the eq. (6) was used according to (Multari, Marsol-Vall, Heponiemi, Suomela, & Yang, 2019):

$$\mathbf{Cox} = [\mathbf{C18} : \mathbf{1} + (\mathbf{10.3} \times \mathbf{C18} : \mathbf{2}) + (\mathbf{21.6} \times \mathbf{C18} : \mathbf{3})] \times \mathbf{100}. \quad (6)$$

Oxidative stability is an important parameter in evaluating the quality of oils and fats, and it is greatly affected by their fatty acid composition and minor components such as tocopherols and phenolic compounds. The oxidation process mainly involves the degradation of polyunsaturated fatty acids and the generation of free radicals, which cause the loss of functional properties and nutritional value (Gordon, Paiva-Martins, & Almeida, 2001).

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

$$\mathbf{OS} = \mathbf{MUFA} + (\mathbf{45} \times \mathbf{C18} : \mathbf{2}) + (\mathbf{100} \times \mathbf{C18} : \mathbf{3}). \quad (7)$$

2.8. Physicochemical properties

According to the American Oil Chemists' Society (Aocs, 1998), certain physicochemical properties are practiced and recommended for vegetable oils, such as the free fatty acid (FFA), specific extinction coefficients (K 232 nm and K 270 nm), peroxide value, iodine value and saponification value at Ca 5a-40, Ch 5–91, Cd 8b-90, Cd 1c-85 respectively.

The FFA was expressed in (% of oleic acid).

The saponification value was expressed in (mg KOH/g oil).

The peroxide value was expressed as (Meq O_2/kg oil).

The iodine value was expressed as (g I_2/100 g oil).

The specific extinction coefficients (K 232 nm and K 270 nm) were expressed as the extinction Specificity of a 1% (*w/v*) solution of oil in cyclohexane.

2.9. Carotenoids and chlorophylls

The vegetable oils dissolved in cyclohexane are measured at 470 nm, where the carotenoids are expressed as mg of lutein per kg of oil, and at 670 nm, the chlorophylls are analysed as mg of pheophytin per kg of oil (Gharby et al., 2018). Therefore, the pigment contents are established using:

$$[\mathbf{Chlorophylls}]\ (\mathbf{mg/kg}) = \mathbf{A_{670}} \times \mathbf{10^6}/\mathbf{613} \times \mathbf{100} \times \mathbf{d}. \quad (8)$$

$$[\mathbf{Carotenoids}]\ (\mathbf{mg/kg}) = \mathbf{A_{470}} \times \mathbf{10^6}/\mathbf{2000} \times \mathbf{100} \times \mathbf{d} \quad (9)$$

'A' = the absorbance

'd' = the thickness of the spectrophotometer cell (1 cm).

The specific extinction coefficients used were $E_0 = 613$ for pheophytin, a major component of the chlorophyll fraction, and $E_0 = 2000$ for lutein, a major component of the carotenoid fraction.

2.10. Statistical analysis

All analyses were performed in triplicate, and the results expressed as the mean ± standard deviation (SD). Data analysis was carried out by one-way ANOVA followed by Tukey's *post hoc* test at $p < 0.05$ using the SPSS version 25.0 for Windows.

3. Results and discussion

3.1. Oil yield

The soxhlet and maceration methods were used to get the oils from the berries of *S. molle* and *S. terebinthifolia*. Table 1 shows the vegetable oil yield for each species.

Despite the differences between extraction methods, the vegetable oil content remains identical for each species; the yield of *S. terebinthifolia* is high, at 13% and then 12.80%, and that of *S. molle* is around 7.6% and then 6.5%, using the soxhlet method and then maceration, successively. Comparing the productivity of the two species, *S. terebinthifolia* is rich in vegetable oil, with a percentage twice then that of *S. molle* by soxhlet (13.3% and 7.64%, respectively) and maceration methods (12.80% and 6.59%, respectively).

Based on the bibliography (Tlili et al., 2018), it was found that the oil yields of *S. terebinthifolia* (14%) were higher than those of *S. molle* (8.5%). Other studies reinforce our results with yields of *S. terebinthifolia* oil per soxhlet using hexane reaching (12.52%), (12.76%) and (14.1%) and (5.11%) (de Oliveira et al., 2020) (De Souza, Arthur, & Nogueira, 2012) (Andrade, Poncelet, & Ferreira, 2017), (Nyakudya et al., 2013), and they found a low yield for *S. molle* (5.35%) (Salhi, Gharsallaoui, & Gabsi, 2021), while the highest yield (22%) is noted for *S. molle* berries from South Africa extracted by maceration for 5 days (Nyakudya et al., 2013).

As *Schinus* and *Pistachia* belong to the same family, Anacardiaceae, and produce berries similar in shape and color, *Schinus* appears to be a cultivated variety while *Pistachia* remains wild. This leads us to compare the results of the species of the two genera. Even the berries of *Pistachia atlantica* and *Pistachia lentiscus* show yields that seem close to those of *Schinus*: 18% (Salhi et al., 2021) and 11.7% (Charef, Yousfi, Saidi, & Stocker, 2008).

Some authors (Bertoldi, 2006; Tlili et al., 2013; Tlili et al., 2014) argue that the variation in yields is influenced by, among other things, environmental factors (climatic, edaphic), variations in the place of cultivation, the degree of maturity, and the variety of the plant.

3.2. Fatty acids composition

The fatty acid composition of the four vegetable oils from the berries of *S. molle* and *S. terebinthifolia* is shown in Table 2.

The percentages of fatty acids do not show any fluctuation when varying the extraction method for each species: saturated fatty acids are around 16% and 20%, monounsaturated fatty acids are 20% and 23%,

Table 1
oil yields of *S. terebinthifolia* and *S. molle* (%).

	S. molle	*S. terebinthifolia*
Soxhlet	7.64 ± 0.03^a	13.3 ± 0.01^b
Maceration	6.59 ± 0.02^a	12.79± 0.02b

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 2
Fatty acids composition of *S. terebinthifolia* and *S. molle* oils (%).

Samples		SMS	SMM	STS	STM
Fatty acid					
Myrsitic acid	C14:0	0.18 ± 0.01[a]	0.19 ± 0.00[a]	0.14 ± 0.00[b]	0.14 ± 0.00[b]
Palmitic acid	C16:0	12.56 ± 0.04[a]	12.4 ± 0.04[a]	15.48 ± 0.1[b]	15.77 ± 0.04[c]
Margaric acid	C17:0	0.29 ± 0.04[a]	0.36 ± 0.02[a]	0.46 ± 0.01[b]	0.37 ± 0.02[a]
Stearic acid	C18:0	2.76 ± 0.16[a]	2.72 ± 0.01[a]	3.49 ± 0.04[b]	3.43 ± 0.07[b]
Arachidic acid	C20:0	0.44 ± 0.04[a]	0.41 ± 0.02[a]	0.36 ± 0.01[b]	0.33 ± 0.02[b]
∑SFA		16.23 ± 0.23[a]	16.08 ± 0.00[a]	19.93 ± 0.12[b]	20.04 ± 0.12[b]
Palmitoleic acid	C16:1	0.69 ± 0.05[a]	0.68 ± 0.01[a]	0.53 ± 0.04[b]	0.63 ± 0.02[a]
Oleic acid	C18:1	19.29 ± 0.02[a]	19.94 ± 0.02[a]	21.69 ± 0.03[b]	23.48 ± 0.04[c]
Ecosenoic acid	C20:1	0.20 ± 0.01[a]	0.19 ± 0.00[a]	0.16 ± 0.01[b]	0.1 ± 0.03[b]
∑MUFA		20.19 ± 0.10[a]	20.83 ± 0.01[b]	22.48 ± 0.03[c]	24.29 ± 0.03[d]
Linoleic acid	C18:2	59.53 ± 0.30[a]	59.29 ± 0.50[a]	55.27 ± 0.63[b]	53.38 ± 0.05[c]
Linolenic acid	C18:3	1.42 ± 0.06[a]	1.34 ± 0.02[a]	2.27 ± 0.02[b]	2.23 ± 0.02[b]
∑PUFA		60.95 ± 0.41[a]	60.63 ± 0.47[b]	57.54 ± 0.67[c]	55.61 ± 0.07[d]
∑UFA		81.14 ± 0.51[a]	81.46 ± 0.48[a]	80.02 ± 0.71[a]	79.9 ± 0.11[b]
ODR		75.61 ± 0.09[a]	75.25 ± 0.61[a]	72.62 ± 0.17[b]	70.31 ± 2.02[b]
LDR		2.32 ± 0.08[a]	2.21 ± 0.17[a]	3.94 ± 0.06[b]	4.01 ± 1.29[b]
DBI		143 ± 0.98[a]	143.43 ± 1.04[a]	139.83 ± 0.93[b]	137.44 ± 3.47[b]

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;
∑UFA; Total unsaturated fatty acids. (∑UFA = ∑MUFA+∑PUFA).
SMM; *S. molle* maceration extract. SMS; *S. molle* soxhlet extract.
STM; *S. terebinthifolia* maceration extract. STS; *S. terebinthifolia* soxhlet extract.

and polyunsaturated fatty acids are 60% and 65%, respectively, for *S. molle* and *S. terebinthifolia*, so the predominance of unsaturated fatty acids is confirmed by the iodine value test that follows the results.

The high percentage of monounsaturated and polyunsaturated fatty acids makes *Schinus* oils suitable for cooking, seasoning, and margarine production (Saber-Tehrani, Givianrad, Aberoomand-Azar, Waqif-Husain, & Jafari Mohammadi, 2013). In addition, they control blood sugar also prevent cardiovascular disease (Donaldson, 2004).

Eleven fatty acids were identified and quantified for both species. Unsaturated fatty acids represented the major part in both *S. terebinthifolia* and *S. molle* (79% and 81%, respectively). Thus, *S. terebinthifolia* contained more saturated fatty acids (20%) than *S. molle* (16%), whereas the latter had more unsaturated (81%) and especially polyunsaturated fatty acids. Important percentages are those corresponding to recent results (Tlili et al., 2018), fifteen fatty acids identified for *S. terebinthifolia* and *S. molle*, with saturated fatty acids appearing toward 31% and 36%, respectively, and unsaturated fatty acids taking the major part (63% - 69%). Essentially, the oil of *S. terebinthifolia* is characterized by more saturated fatty acids, while *S. molle* presents more unsaturated fatty acids. Qualitatively, the results obtained from this study were in agreement with previously reported information (Ennigrou et al., 2017; Hosni et al., 2011).

The differences found may be due to genetic variability, as has been reported for other phytochemicals of these species (Tlili et al., 2018), as well as climatic conditions and berries maturity (Breene, Lin, Hardman, & Orf, 1988; Tlili et al., 2014).

Specifically, the majority compounds of the eleven fatty acids identified in the four berries oils successively from *S. molle* and *S. terebinthifolia* are linoleic acid (59.53% and 55%; C18:2), oleic acid (19.29% and 21.69%; C18:1), palmitic acid (12.56% and 15.48%; C16:0), stearic acid (2.76% and 3.50%), and palmitoleic acid (0.69% and 0.53%; C16:1). This fatty acid profile is similar to that in the literature (de Oliveira et al., 2020; Ennigrou et al., 2017) which focused in particular on the *S. terebinthifolia* species, and is more interesting than that of SMM (Nyakudya et al., 2013). While the composition differs for *P. atlantica* vegetable oil, which is characterized by its high abundance of oleic acid (56.35%), followed by linoleic acid (28.74%), then palmitic acid (4.45%), and palmitoleic (acid 11.16%) (Gharsallaoui et al., 2017.; Salhi et al., 2021),

Both methods rely on the high activity of the desaturation of oleic acid (ODR). On the other hand, there is a decrease in the values of DBI. This opposition is a good explanation for the low content of linolenic acid, a fact that is affirmed by the lowering of the rate of linolenic desaturation (LDR). The results are in agreement with those reported, specifying the species of *S. terebinthifolia* (Ennigrou et al., 2017) and also discussing the species *S. molle* (Hosni et al., 2011).

3.3. Nutritional indexes

The calculated oxidability value (Cox), oxidative susceptibility (OS), the UFA/SFA ratio, the (ω3/ω6) ratio, the atherogenic index (AI), and the thrombogenic index (TI) were studied for the red berries extracts (Table 3).

The UFA/SFA ratio remained stable after changing the extraction method. The values obtained for *S. molle* and *S. terebinthifoli*a (0.2–0.25) were much lower than those of 3.05 (Ennigrou et al., 2017). Similarly, for the (ω3/ω6) ratio, a low value of 0.02 for *S. molle* and 0.04 for *S. terebinthifolia* was observed. The recommendations state that the (ω3/ω6) ratio in the human diet should be below 0.02 (Jankowska, Zakęś, Żmijewski, & Szczepkowski, 2010). Therefore, the red berries of *S. terebinthifolia* and *S. molle* meet this requirement and could be considered suitable for human nutrition. This assumption is supported by the Cox value of 6 for all samples and the values (2800 and 2700) of OS for *S. molle* and *S. terebinthifolia*, which are identical to the values that show that these oils are less vulnerable to oxidation (Ennigrou et al., 2017). Other positive properties added to the profiles of these lipids are AI and TI, with SM samples showing 0.004 and 0.007 for AI and TI respectively, and ST oils showing approximately 0.006 for AI and 0.01 for TI, these results remain low to the high values found such as 0.31 for AI and 0.44 for TI (Ennigrou et al., 2017). According to the study (Hosseini et al., 2014), the low values of AI and TI for both species correlate well with the reduced risk of coronary heart disease.

Summarizing, the nutritional quality indexes claimed the healthiest attributes of the ripe red berries of *S. molle* and *S. terebinthifolia* with both extraction methods.

Table 3
Nutritional indexes of *S. terebinthifolia* and *S. molle* oils.

Samples / Indexes	SMS	SMM	STS	STM
UFA/SFA	0.2	0.19	0.25	0.25
ω3/ω6	0.024	0.023	0.041	0.042
Cox	6.63 ± 0.04[a]	6.66 ± 0.07[a]	6.40 ± 0.04[b]	6.21 ± 0.18[b]
OS	2841.04 ± 22.06[a]	2822.88 ± 34.90[a]	2736.63 ± 19.77[b]	2649.39 ± 80.76[b]
AI	0.004 ± 0.00[a]	0.004 ± 0.00[a]	0.005 ± 0.00[a]	0.006 ± 0.00[a]
TI	0.007 ± 0.00[a]	0.007 ± 0.00[a]	0.009 ± 0.00[a]	0.01 ± 0.00[a]

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
Phytosterol composition of *S. molle* and *S. terebinthifolia* oils (mg/kg).

Samples / phytosterols	SMS	SMM	STS	STM
Cholesterol	39.82 ± 0.05^{a} (1.74%)	11.28 ± 0.02^{b} (1.04%)	54.77 ± 0.09^{c} (2.75%)	40.41 ± 0.05^{a} (2.03%)
Campesterol	179.66 ± 0.007^{a} (7.85%)	63.48 ± 0.00^{b} (5.85%)	170.10 ± 0.02^{c} (8.54%)	138.41 ± 0.01^{d} (7.03%)
Stigmasterol	37.30 ± 0.04^{a} (1.63%)	15.35 ± 0.04^{b} (0.77%)	54.59 ± 0.014^{c} (3.96%)	34.86 ± 0.02^{a} (3.16%)
β-sitosterol	3840.19 ± 0.02^{a} (69.35%)	2566.96 ± 0.01^{b} (46.35%)	3907.66 ± 0.02^{c} (70.57%)	3520.25 ± 0.41^{d} (63.57%)
Δ-5-avenasterol	117.86 ± 0.03^{a} (5.15%)	34.50 ± 0.00^{b} (3.18%)	136.64 ± 0.00^{c} (6.86%)	80.75 ± 0.07^{d} (5.07%)
Δ-7-stigmasterol	46.12 ± 0.15^{a} (1.74%)	39.18 ± 0.02^{b} (0.57%)	51.58 ± 0.02^{c} (2.59%)	37.65 ± 0.00^{b} (0.68%)
Δ-7-avenasterol	76.67 ± 0.04^{a} (3.35%)	46.51 ± 0.00^{b} (0.84%)	99.99 ± 0.01^{c} (4.02%)	53.16 ± 0.07^{d} (0.96%)
Total (mg/kg)	4337.62 ± 0.55^{a}	2777.26 ± 0.0^{b}	4475.33 ± 0.05^{c}	3905.49 ± 0.69^{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 ($p < 0.05$).
SMM; *S. molle* maceration extract. SMS; *S. molle* soxhlet extract.
STM; *S. terebinthifolia* maceration extract. STS; *S. terebinthifolia* soxhlet extract.

3.4. Phytosterol composition

The Phytosterol composition analyses of the four oils obtained showed that the dominant sterol is *β*-sitosterol, followed by campesterol, Δ-5-avenasterol, and Δ-7-avenasterol. The *β*-sitosterol varies according to the species and the extraction method and remains the dominant compound since it is 69%, 46%, 70%, and 63% for SMS, SMM, STS, and STM, respectively (Table 4). The differences between the two species could be attributed to variations in the structure and chemical composition of the extracts (Liu et al., 2019). In general, phytosterols are influenced by the extraction method, resulting in differences in percentage and concentration for *S. molle* and *S. terebinthifolia*.

The two species and extraction methods show a significant difference in the amount of phytosterols they contain. *S. molle* oils contain lower concentrations of phytosterols than *S.terebinthifolia* oils. Total phytosterols demonstrate 4337 mg/kg (SMS), 2777 mg/kg (SMM), 4475.33 mg/kg (STS) and 3905 mg/kg (STM). This distinction between methods is made clear by analysis of the concentrations of each compound, *β*-sitosterol, which varies from 2566 to 3840 for SMM and SMS, and from 3520 to 3907 for STM and STS, campesterol shows high concentrations, especially in oils extracted by the soxhlet method, SMS (179 mg/kg) and STS (170 mg/kg), then Δ-5-avenasterol which shows a remarkable difference in concentrations in both species SMS = 117 mg/kg, SMM = 34 mg/kg, and STS = 136 mg/kg, STM = 80 mg/kg), similarly Δ-7-avenasterol reveals a variation in SMS = 76 mg/kg, SMM = 46 mg/kg, and STS = 99 mg/kg, STM = 53 mg/kg. Which proves the influence of the extraction method on phytosterol content.

In terms of *β*-sitosterol, the four oils showed an approximation to the *P. atlantica* oils (85%, 87%, and 87%) (Benhassaini, Bendahmane, & Benchalgo, 2007; Saber-Tehrani et al., 2013; Yousfi, Nedjmi, Bellal, Bertal, & Palla, 2002), while, a low content of *β*-sitosterol corresponds to 1650 mg/kg and 1665 mg/kg) is compared with the concentrations of SMS, STS, and STM samples (3840, 3907, and 3520 mg/kg respectively) (Salhi et al., 2021; Tavakoli et al., 2019), campesterol occupies almost the same percentage for STS (8.54%) and SMS (7.85%), while maceration indicates low and uneven concentrations for STM (7.03%) and SMM (5.85%), and remain high compared with (4%) other studies (Benhassaini et al., 2007; Yousfi et al., 2002).

In terms of concentrations (mg/kg), SMS, STS and STM also showed wide variations between 179 mg/kg, 170 mg/kg and 138 mg/kg, and *P. atlantica* concentrations of 87 mg/kg and 77 mg/kg. (Salhi et al., 2021; Tavakoli et al., 2019), the authors also noted significantly higher cholesterol levels (59 mg/kg, 49 mg/kg) compared to our results obtained with *S. molle* (39 mg/kg, 11 mg/kg).

Table 5
Tocopherol content of *S. molle* and *S. terebinthifolia* oils (mg/kg).

	SMS	SMM	STS	STM
α-tocopherol	74.02 ± 0.10^{a}	58.33 ± 0.10^{b}	77.34 ± 0.10^{c}	62.51 ± 0.10^{d}
β-tocopherol	150.68 ± 0.43^{a}	103.24 ± 0.22^{b}	309.56 ± 0.50^{c}	220.04 ± 0.47^{d}
γ-tocopherol	352.11 ± 0.06^{a}	266.02 ± 0.10^{b}	506.30 ± 0.20^{c}	483.10 ± 0.15^{d}
δ-tocopherol	58.11 ± 0.13^{a}	54.30 ± 0.21^{b}	64.18 ± 0.52^{c}	61.44 ± 0.27^{d}
Total tocopherols (mg/kg)	674.92 ± 0.25	501.56 ± 0.07	988.38 ± 0.29	857.09 ± 0.01

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 ($p < 0.05$).
SMM; *S. molle* maceration extract. SMS; *S. molle* soxhlet extract.
STM; *S. terebinthifolia* maceration extract. STS; *S. terebinthifolia* soxhlet extract.

3.5. Tocopherols composition

Tocopherols are the main components of unsaponifiable matter in vegetable oils that have antioxidant activities, are mentioned in the form

Table 6
The physicochemical properties of *S. molle* and *S. terebinthifolia* oils.

	SMS	SMM	STS	STM
FFA (%)	2.115 ± 0.0039^{a}	2.588 ± 0.0039 ^{a}	1.934 ± 0.0279 ^{b}	1.849 ± 0.0159 ^{b}
PV (meq O_2/kg)	52 ± 1.414^{a}	49 ± 8.485^{a}	105 ± 7	92.5 ± 3.535^{c}
SV (mg KOH/g)	2691.06 ± 270.21^{a}	2574.031 ± 376.22^{a}	2043.96 ± 223.39^{b}	2233.20 ± 332.62 ^{b}
IV (g I_2/100g oil)	144.62 ± 0.05a	144.46 ± 0.06 ^{a}	144.54 ± 0.00 ^{a}	143.10 ± 0.04 ^{a}
K 232 nm	0.071 ± 0.006 ^{a}	0.039 ± 0.006^{b}	0.042 ± 0.003 ^{b}	0.025 ± 0.007 ^{b}
K 270 nm	0.216 ± 0.008 ^{a}	0.221 ± 0.010 ^{a}	0.219 ± 0.007 ^{a}	0.207 ± 0.011 ^{a}
Chlorophylls (mg/kg)	0.448 ± 0.015^{a}	0.497 ± 0.075^{a}	0.840 ± 0.095^{b}	0.399 ± 0.065^{a}
Carotenoids (mg/kg)	1.512 ± 0.05^{a}	2.020 ± 0.12^{b}	1.61 ± 0.01^{a}	0.787 ± 0.016^{c}

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 ($p < 0.05$).
SMM; *S. molle* maceration extract. SMS; *S. molle* soxhlet extract.
STM; *S. terebinthifolia* maceration extract. STS; *S. terebinthifolia* soxhlet extract.

of active vitamin E, and are essential for human health. Table 5 summarizes the tocopherol content of the oils extracted by the two methods.

The content of tocopherol isomers varies significantly depending on extraction methods and species. Γ-tocopherol and β-tocopherol are the main isomers identified with high contents for all oils, such as STS (506–309 mg/kg), and STM (483–220 mg/kg), SMS (352–150 mg/kg), SMM (266–103 mg/kg) respectively, so maceration gives less tocopherol content than soxhlet and *S. terebinthifolia* oils are rich in tocopherols than those of *S. molle*.

P. atlantica berries oil shares the same main component γ-tocopherol with *schinus* species but with a slightly low content of 425.56 mg/kg and 302 mg/kg lower than STS, STM and SMS and close of SMM, respectively (Salhi et al., 2021) (Tavakoli et al., 2019), and for other isomers, such as α- and β-tocopherols, are present in quantities of (64.85 mg/kg, 130.18 mg/kg) and (74 mg/kg, 140 mg/kg) respectively(Salhi et al., 2021) (Tavakoli et al., 2019).

3.6. Physicochemical properties

The physicochemical properties of the four *Schinus* oils extracted by the two methods are gathered and quoted in Table 6.

Free fatty acid (FFA); saponification value (SV); peroxide value (PV); iodine value (IV); specific Extinction coefficients (K 232 and K 270).

The berries oils studied do not show interesting variability in terms of species and extraction method. However, the percentage of acidity is much higher in *S. molle* than in *S. terebinthifolia*, ranging from 1.9% to 2.5%. These high values can be attributed to the hydrolysis of triglycerides or even increased free fatty acid functions in the oils, with an unusually high acid value (178.23 ± 36.8 mg KOH/g oil) of SMM oil (Nyakudya et al., 2013). Meanwhile a percentage acidity of (4.26% ± 0.085) for *P. atlantica* berries and (2.15 ± 0.017 meq O_2/kg) for the peroxide value is notably low than the *Schinus* species (Salhi et al., 2021). Since red seeds are not hulled before crushing, the polyphenols present in the seed shells can possibly cause high acidity values (Nyakudya et al., 2013).

The peroxide analysis carried out in the present study indicates that the oil extracted by maceration has the lowest peroxide value (49 ± 8.485 meq O_2/kg for SMM and 92.5 ± 3.535 meq O_2/kg for STM). This compares with the high values recorded for the oils extracted by the soxhlet method (52 ± 1.414 meq O_2/kg for SMS and 105 ± 7.071 meq O_2/kg for STS). Also, it is noted that the soxhlet extraction process influences the formation of secondary oxidation products, resulting in soxhlet-extracted oil being more oxidized, which may shorten its storage time, according to analyses of *P. atlantica* (Salhi et al., 2021).

The specific extinction at 232 nm and 270 nm reflects the oxidation state of the oils, as the absorbance at 232 nm shows the existence of primary oxidation products and indicates that the oil is peroxidized, and the absorbance at 270 nm reflects the formation of secondary oxidation products. Extinction at 232 nm shows no significant change in the oils of both species, but the soxhlet method shares the higher values (*S. molle* = 0.071, *S. terebinthifolia* = 0.042). There is an expressive change in the oxidation state, such that the absorbances show 0.071, 0.039, 0.042, and 0.025 at 232 nm and 0.216, 0.221, 0.219, and 0.207 at 270 nm for SMS, SMM, STS, and STM respectively. And compared to *P. atlantica*, the results indicate 2.17 at 232 nm and 0.95 at 270 nm (Gharsallaoui et al., 2017.; Salhi et al., 2021).

The low iodine number of saturated oils favors the drying properties of the oils since they are not exposed to oxidation by forming a dry and hard film (Essien, Umoren, Essien, & Udoh, 2012). The relatively high iodine number in the four oils may indicate the presence of many unsaturated bonds, and they certainly contain more unsaturated fatty acids, meaning they can be grouped into drying oils (Charef et al., 2008). This is the case for the iodine value of *Schinus* oils: the four have a value of 144 g I_2/100g of oil, contrary to the results (Nyakudya et al., 2013). SMM oil (17.74 g I_2/100g oil) has a drying property, making it a potential candidate as a base for the manufacture of paints and varnishes. Furthermore, the high oxidative stability of the oil makes it safe for cooking and for use as a dietary supplement. The chemical examinations of *P. lentiscus* oils reported are in agreement with our results (Charef et al., 2008).

The saponification index is 2882.138 mg KOH/g (SMS), 2840.063 mg KOH/g (SMM), 2201.925 mg KOH/g (STS) and 2468.4 mg KOH/g (STM), it there is therefore no considerable difference between the values. These red berries are distinct from the recently reported macerated *S. molle* berries which contain 129.88 mg KOH/g oil (Nyakudya et al., 2013) and the latter are close to the red berries of *P. lentiscus* (154 0.6 and 130.5 mg KOH/g) (Charef et al., 2008). High saponification oils are used as ingredients in soap making, shaving foam and cosmetics (Nzikou et al., 2007; Thomas, Matthäus, & Fiebig, 2000).

3.7. Chlorophyll and carotenoid pigments

In the dark, chlorophylls act as antioxidants that protect cells from free radical damage, so greenish oils should be stored in the dark (Donaldson, 2004). Carotenoids are the pigments responsible for the yellow-orange color of oils and have an antioxidant power (Bai et al., 2016). Among the carotenoids, vitamin A, the precursor of β-carotene, has provitamin activity, making it a good element for incorporation into functional foods (Boon, McClements, Weiss, & Decker, 2010).

The quantitative and qualitative composition of chlorophyll pigments and carotenoids differs between *Schinus* oils. Chlorophyll content shows 0.448 mg/kg and 0.497 mg/kg for SMS and SMM respectively, while for STS and STM the content becomes 0.840 mg/kg and 0.399 mg/kg. Similarly, carotenoid content shows 1.512 mg/kg and 2.020 mg/kg for SMS and SMM respectively, and for STS and STM reveals 0.787 mg/kg and 1.61 mg/kg respectively.

This proves that these two species behave in an approximate way except that soxhlet extraction yields double the amount of chlorophyll from *S. terebinthifolia*, and maceration makes *S. molle* react better to release enough carotenoids. For carotenoids, a total content of 24.66 μg/g and 27.5 μg/g are determined for *S. terebinthifolia* berries (de Oliveira et al., 2020; Pagani et al., 2014). The Tunisian *P. atlantica* berries we got using the soxhlet method have a chlorophyll level about the same as ours (0.75 mg/kg), but they have a lot more carotenoids than ours (20.29 mg/kg) (Salhi et al., 2021).

4. Conclusion

S. terebinthifolia and *S. molle* berries oils share a similar phytochemical profile, characterized by the high content of linoleic acid, α-tocopherol, and β-sitosterol. Nutritionally, the indexes claim the healthiest attributes of ripe berries oils. The desaturation levels and physicochemical properties show the interesting stability of the oils which is proven by the carotenoid and chlorophyll content. Consequently, soxhlet extraction influences the oil quality by reducing phytosterols and tocopherols content and increasing acidity, peroxide value and primary oxidation products.

CRediT authorship contribution statement

Oumayma Belhoussaine: Writing – original draft, Resources, Methodology, Data curation. **Chaimae El Kourchi:** Writing – original draft, Investigation, Data curation. **Hicham Harhar:** Writing – review & editing, Supervision, Conceptualization. **Hamza El Moudden:** Writing – review & editing, Software, Resources, Investigation. **Adil El Yadini:** Writing – review & editing, Validation, Methodology, Investigation. **Riaz Ullah:** Writing – review & editing, Investigation, Funding acquisition. **Zafar Iqbal:** Writing – review & editing, Software, Funding acquisition, Formal analysis. **Khang Wen Goh:** Writing – review & editing, Resources, Methodology, Funding acquisition. **Bey Hing Goh:** Writing – review & editing, Visualization, Investigation, Funding acquisition, Data curation. **Abdelhakim Bouyahya:** Writing – review &

editing, Supervision, Formal analysis. **Mohamed Tabyaoui:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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