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Journal of Oleo Science

doi : 10.5650/jos.ess22110
J. Oleo Sci. **71**, *(8) 1117-1133 (2022)*

JOURNAL OF OLEO SCIENCE
Jos

Profiles of Fatty Acids, Polyphenols, Sterols, and Tocopherols and Scavenging Property of Mediterranean Oils: New Sources of Dietary Nutrients for the Prevention of Age-related Diseases

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Abstract: The present study provides the fatty acid, tocopherol, phytosterol, and polyphenol profiles of some Mediterranean oils extracted from pumpkin, melon, and black cumin seed oils and those of dietary argan seed oil. Gas chromatography analysis revealed that oleic and linoleic acids were the most abundant fatty acids. Argan and melon seed oils exhibited the highest levels of oleic acid (47.32±0.02%) and linoleic acid (58.35±0.26%), respectively. In terms of tocopherols, melon seed oil showed the highest amount (652.1±3.26 mg/kg) with a predominance of γ-tocopherol (633.1±18.81 mg/kg). The phytosterol content varied between 2237.00±37.55 µg/g for argan oil to 6995.55±224.01 µg/g for melon seed oil. High Performance Liquid Chromatography analysis also revealed the presence of several polyphenols: vanillin (0.59 mg equivalents Quercetin/100 g) for melon seed oil, and p-hydroxycinnamic acid (0.04 mg equivalents Quercetin/100 g), coumarine (0.05 mg equivalents Quercetin/100 g), and thymoquinone (1.2 mg equivalents Quercetin/100 g) for black cumin seed oil. The "Kit Radicaux Libres" (KRL) assay used to evaluate the scavenging properties of the oils showed that black cumin seed oil was the most efficient. On the light of the richness of all Mediterranean oil samples in bioactive compounds, the seed oils studied can be considered as important sources of nutrients endowed with cytoprotective properties which benefits in preventing age-related diseases which are characterized by an enhanced oxidative stress.

Key words: Mediterranean oils, nutrients, fatty acids, tocopherols, phytosterols, polyphenols, antioxidant activity

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Accepted April 20, 2022 (received for review March 26, 2022)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

1 Introduction

Plants have acquired and developed the ability to synthesize thousands of metabolites with various biological activities. Increasing knowledge about the nutraceutical role of these substances has major implications for plants in pharmacy, cosmetics, and nutrition. Nutraceuticals are plant food derivatives that have medical benefits, such as the prevention and treatment of diseases. These benefits may be reflected in free radical scavenging activities, metal chelating properties and singlet oxygen quenching effects[1]. Naturally occurring antioxidants have shown beneficial activities on human health, including reduced DNA damage, mutagenesis, and carcinogenesis. The organoleptic, nutritional and nutraceutical properties of plant foods are defined by a complex mix of characteristics, mainly attributable to their complex composition. Plant genetics, environmental parameters, and agronomic factors, such as geographic area, stage of maturity at harvest, cultural practices, soil nutritional conditions and cultivation methods strongly influence the synthesis and storage of these substances by plants[2].

Natural products are generally divided into two families: primary and secondary metabolites. Primary metabolites are widely represented in nature and include a wide variety of compounds: vegetable oils, fatty acids, and carbohydrates (starch, pectin and cellulose)[3]. Secondary metabolites (phenolic compounds, terpenoids) are formed from primary metabolites, the majority of which are not directly involved in growth and development[4].

In the Mediterranean basin, several vegetable oils are known to have health benefits such as argan oil, black cumin, pumpkin, melon and milk thistle seed oils. Some of these oils (argan oil, olive oil, and milk thistle seed oil) have been shown to prevent the cytotoxic activities of cholesterol oxide derivatives (also named oxysterols) which can be found at high levels in patients with major age-related diseases (cardiovascular diseases, neurodegenerative diseases, eyes diseases, and sarcopenia)[5, 6]. Argan oil (AO) (*Argania spinosa* L.) is nowadays used in traditional medicine in Morocco. In this country, in the 'Amazigh' diet, argan oil is also used as a traditional food ingredient, bringing almost 25% of total diet fat intake to indigenous consumers[7]. It is a virgin oil obtained using a cold-pressing technique avoiding any changes in its composition during the extraction procedure[8]. Furthermore, any refining stage is part of the argan oil preparation process. In terms of chemical composition, argan oil was reported to be homogenous and unaffected by geographical or genetic factors[9]. Argan oil's therapeutic benefits are increasingly sought after these days due to its unique lipophilic antioxidant composition, which consists primarily of monounsaturated (up to 80%) and saturated (up to 20%) fatty acids, tocopherols, sterols, and polyphenols[10]. In addition to being safe, this unique blend of lipophilic compounds has been linked to the prevention of noncommunicable diseases, now considered as civilization diseases and mainly resulting from life habits, pollution and environmental stress, such as cardiovascular disease, hypercholesterolemia, and obesity, as well as the treatment of many dermatological disorders[11]. The neuroprotective properties of AO were also proven by using both nerve cell lines and different neurodegenerative animal models[10, 12, 13].

Black cumin (*Nigella sativa*) oil and seeds have been widely used for centuries to treat a variety of diseases and are regarded as an important drug in traditional medical systems in Asian and Middle Eastern countries (Ayurveda, Unani, Arabic, and Chinese medicines)[14]. Thanks to its richness in phytochemicals (essential fatty acids, phenolic compounds, tocopherols, sterols), *Nigella sativa* seed oil (NSO) is of particular interest. Besides these biocomponents, thymoquinone (TQ) in high levels (460-873 mg/100 g oil) is considered as a bioactive compound endowed with an interesting antioxidant activity[15]. Ramadan *et al.*[16] assessed the antioxidant activity of NSO and compared it with olive oil using stable DPPH. (1,1-diphenyl-2-picrylhydrazyl) and galvinoxyl radicals. The two radical systems showed that NSO had higher radical scavenging activity than olive oil. With regards to the health benefits of NSO, such unconventional oil resource was observed to decrease serum cholesterol level by 15.5% and triglyceride level by 22% in normal rats[17]. In this context, the effects of black cumin on the lipid profile were thought to be the result of a combination of various components, such as TQ, sterols, and flavonoids, rather than any single component[18]. Otherwise, the oil and aqueous extract of black cumin were proven both effective at controlling serum glucose and insulin response. A number of studies have shown that black cumin extract/oil consumption has a positive effect on parameters such as serum insulin, super dismutase (SOD), serum glucose, and malondialdehyde (MDA) levels[14]. It is worth noting that NSO was also efficient in reducing in a significant manner the viability of human lung cancer cells suggesting that the anticancer activity might be linked to the presence of active compounds[19]. It has also been recently reported that whole and purified aqueous extracts of black cumin seeds attenuate apoptosis and the overproduction of reactive oxygen species (ROS) triggered by p53 over-expression in the yeast *Saccharomyces cerevisiae*[20].

Pumpkin (*Cucurbita* sp.) and melon (*Cucumis melo* L.) are regarded as the main important species of the Cucurbitaceae family which is a medium-sized plant family growing extensively in temperate, tropical and subtropical regions of the world[21]. *Cucurbita* sp. includes many varieties such as *Cucurbita maxima*, *Cucurbita pepo*, *Cucurbita moschata*, *Cucurbita ficifolia*, and *Cucurbita turbaniformis*[22]. Thanks to their unique composition, seeds are considered the most important component of the

pumpkin. Besides their high fat and protein contents with essential amino acids, pumpkin seed oil (PSO) contains substantial amounts of bioactive compounds such as ω-3, -6 and -9 fatty acids, α- and γ-tocopherols, sterols, β-carotene and lutein[23]. Thanks to these biocomponents, PSO was reported to possess diverse health benefits. In fact, Nishimura *et al.*[24] demonstrated that *Cucurbita maxima* seed oil was effective in preventing urinary disorders such as overactive bladder (OAB) and benign prostate hypertrophy (BPH). These effects were attributed to the oil's sitosterol content (belonging to the phytosterols family known for their health benefits[25], indicating its potential in the prevention or treatment of urinary disorders such as OAB. Bharti *et al.*[26] also found pharmacological evidence that the tocopherol fraction of *Cucurbita pepo* L. raw seeds has an anti-hyperglycemic effect mediated by interactions of its different components with several signaling pathways that are important in diabetes mellitus (DM). In the same context, an *in vivo* investigation reported that the high level of tocopherols, sterols, and polyunsaturated fatty acids in PSO was associated with effective wound healing. In fact, unlike the untreated group, morphometric analysis and histological findings indicated healed biopsies from the pumpkin oil-treated rats, as well as a complete re-epithelialization with the return of skin appendages and well-organized collagen fibers free of inflammatory cells[27].

Melon (*Cucumis melo* L.) seeds could be used for the plant oil extraction since they are considered as a rich source of biocomponents and natural antioxidants such as ω-6 and ω-9 fatty acids, α- and γ-tocopherols, and sterols[28]. It is noteworthy that information related to bioactive compounds in melon seed oil is scarce. Nakai (Kalahari melon) seed oil was researched for its ability to preserve good skin. In reality, melon seed oil includes a variety of fatty acids that may be useful when applied to the skin topically. Komane *et al.*[29] investigated the safety (irritancy potential test) and effectiveness (transepidermal water loss, hydration, and occlusivity tests) of topically administered Kalahari melon seed oil on healthy Caucasian adult female volunteers (n = 20). When used topically, Kalahari melon seed oil did not irritate the skin (observed at 24, 48, 72 and 96 h). The irritancy level findings demonstrated that using Kalahari melon seed oil decreased transepidermal water loss and boosted moisture retention. The non-irritant, hydrating, and moisturising properties of Kalahari melon seed oil demonstrated in this research support its use at cosmetic scale.

Thanks to their richness in numerous bioactive compounds such as tocopherols, phytosterols and polyphenols, several Mediterranean oils can have protective activities both at the level of oxidative stress and at the level of organelles (mitochondria, peroxisomes, lysosomes) to prevent their dysfunctions[6]. Indeed, some of these compounds such as tocopherols and polyphenols have mitotherapeutic and pexotherapeutic properties (attenuation of mitochondrial and peroxisomal damages, respectively) and are important in maintaining the redox equilibrium and controlling mitochondrial activity: two essential parameters required for an optimal cell activity. There are now several studies supporting that the nutrients present in Mediterranean oils may help to prevent age-related diseases: cardiovascular diseases as well as some eye and neurodegenerative diseases (cataract, age related macular degeneration, Parkinson's diseases, Alzheimer's diseases, sarcopenia)[30–32]. These different diseases are characterized by an increased oxidative stress and the occurrence of organelle damage especially mitochondrial dysfunctions[33–35]. As several compounds present in the oils can cross the blood brain barrier, some oils may have a great interest to prevent and/or treat neurodegenerative diseases whose frequency increases with age worldwide. Neurodegenerative diseases are a serious public health problem, and no efficient treatments are currently available[36]. So, it is important to identify natural or synthetic molecules or mixture of molecules (such as oils) to prevent the appearance or to stop the evolution of age-related diseases. Such attempts will permit to prevent or reduce the incidence of age-related diseases which are increasing in most countries due to the enhancement of life expectancy[37].

In line with the current nutrition trends encouraging the use of functional foods endowed with interesting medicinal benefits, the present study was conducted to identify new sources of Mediterranean vegetable oils containing bioactive compounds with antioxidant and cytoprotective properties. For these reasons, we were interested by the nutrient profile of the following Mediterranean oils (argan, pumpkin, melon, and black cumin oils) used in Mediterranean cuisine.

The aim of the present study consisted of establishing the fatty acids, polyphenols, tocopherols and phytosterols profiles and the scavenging properties of pumpkin, melon, and black cumin seed oils as well as argan oil in order to enhance public awareness about the Mediterranean seed oil's potential health advantages and to pique researchers' interest in exploring their potential health benefits particularly on aging and on the prevention of age-related diseases.

2 Material and Methods

2.1 Oil samples origins and preparation

Seeds of pumpkin (*Cucurbita pepo*) of variety 'Essahli', (*Cucurbita maxima*) of variety 'Béjaoui', and of melon (*Cucumis melo*) of variety 'Ananas' were studied. Approximately 4 kg of each Cucurbitaceae variety were bought from a local market in Chebika region (latitude 35° 37'38''; longitude 10° 2.15'38''; elevation 86 m), situated in

west-central Tunisia. *Nigella sativa* seeds (commonly called black cumin) were also used. Roughly 4 kg of seeds were purchased from souk Blat (Tunisia). The seeds were first selected, and only those which were entirely and totally formed were used, washed to eliminate impurities, and finally air-dried for 48 h in a forced circulation oven at 40 ± 0.5℃ and stored in glass jars.

Cold pressing is performed three times by pressing raw/dried seeds directly at a low temperature on a continuous screw press. Notice that in the current study the *Cucurbita maxima* var. 'Béjaoui' pumpkin seed oil was mechanically obtained using a MUV2 65 vegetable oil screw press (Smir Technotour, Agadir, Morocco), named as PSO1 and a Komet DD 85 G screw press (IBG MonfortsOekotec GmbH & Co. KG, Monchengladbach, Germany), named as PSO2. *Cucurbita pepo* var. 'Essahli', *Cucumis melo* var. 'Ananas', and *Nigella sativa* seed oils were mechanically obtained using a Komet DD 85 G screw press (IBG Monforts Oekotec GmbH & Co. KG, Monchengladbach, Germany). The latters were named respectively as PSO3, MSO, and NSO.

Due to the fact that the technology of cold pressing oil excludes any material thermal processing during the preparation phase, this may induce some difficulties in the initial stage of using a screw press. Before starting the pressing process, the head of the screw press is heated to a temperature ranging from 60 to 70℃ in order to reduce danger and achieve higher efficiency. For this reason, the head of the screw press was attached to a specially designed heating ring, connected to an automatic temperature control device. The temperature of the extracted oil is not influenced by head preheating since the heaters are always turned off when the optimum seed flow has already been attained[23]. The extracted oil was subsequently separated from the sediment at 5,000 rpm by centrifugation (Hettich Zentrifugen, model Universal 32R) for 10 min at 24℃ and preserved in a freezer at −20℃.

Biological and unrefined argan oil and pumpkin (*Cucurbita pepo* Subsp. *Pepo*) of variety "Styriaca" seed oils, named PSO4 and AO, respectively, were purchased from "Biocoop" society (Paris, France) with expiration dates ≥ 6 months. They were stored at −20℃ for further analysis within their expiration dates. Argan oil, which was frequently analyzed in our laboratory, was used as control in order to validate the values of the different nutrients measured (fatty acids, polyphenols, tocopherols, and phytosterols). We also choose to include a commercial pumpkin oil in the study to assess whether melon and pumpkin oils prepared from Tunisian seeds may vary compared to commercial standards.

All oil samples concerned by the present study with their respective abbreviations are illustrated in Table 1.

2.2 Fatty acid analysis by gas chromatography

Lipids were extracted from the different oils according to the Moilanen & Nikkari[38] method. C19:0 was used as internal standard. Fifteen mg of lipids were submitted in accordance with Morrison & Smith[39] to fatty acid methylation using 7% boron trifluoride in methanol. The fatty acid methyl esters (FAMEs) were subsequently analyzed using a TRACE 1310 (Thermo Scientific) gas chromatograph using a CPSIL-88 column (100 m × 0.25 mm internal diameter (internal diameter: i.d.)), 0.2 μm film thickness (Varian, Les Ulis, France) equipped with a flame ionization detector (FID). Hydrogen was used as the carrier gas (inlet pressure, 210 kPa). The oven temperature was kept at 60℃ for 5 min, increased to 165℃ at 15℃/min and held for 1 min, and then to 225℃ at 2℃/min and finally held at 250℃ for 17 min. The injector and the detector were held at 250℃ and 280℃, respectively. FAMEs were identified by comparison with commercial and synthetic standards (Sigma Aldrich). The data were processed using the ChromQuest

Table 1 Analyzed oil samples with their respective abbreviations.

Oils	Abbreviation
Cucurbita maxima var. 'Béjaoui' pumpkin seed oil obtained using a MUV2 65 screw press (Smir Technotour, Agadir, Morocco)	PSO1
Cucurbita maxima var. 'Béjaoui' pumpkin seed oil obtained using a Komet DD 85 G screw press (IBG Monforts Oekotec GmbH & Co. KG, Mönchengladbach, Germany)	PSO2
Cucurbita pepo var. 'Essahli' seed oil obtained using a Komet DD 85 G screw press (IBG Monforts Oekotec GmbH & Co. KG, Mönchengladbach, Germany)	PSO3
Cucumis melo var. 'Ananas' seed oil obtained using a Komet DD 85 G screw press (IBG Monforts Oekotec GmbH & Co. KG, Mönchengladbach, Germany)	MSO
Nigella sativa seed oil obtained using a Komet DD 85 G screw press (IBG Monforts Oekotec GmbH & Co. KG, Mönchengladbach, Germany)	NSO
Commercial *Cucurbita pepo* Subsp. *Pepo* var. 'Styriaca' seed oil	PSO4
Commercial argan oil (Emile Noël / maître moulinier, Biocoop, France)	AO

software (Thermo Scientific) and reported as a percentage relative to total fatty acids (considered as 100%).

2.3 Tocopherol analysis by high performance liquid chromatography (HPLC)

One hundred mg of oil was resuspended in 1 mL of hexane and diluted to 1/10 in mobile High Performance Liquid Chromatography (HPLC) phase: methanol/TBME/water (700:280:20, v/v/v). After resuspension, the extract was vortexed for 30 s. Samples of 10 μL were injected into the HPLC system for the analysis of tocopherols. The HPLC apparatus was a Dionex U3000 equipped with an automatic injector system and a Dionex multi-wavelength detector. HPLC analyses were carried out using a C30 YMC carotenoid column (250 × 2.1 mm i.d., 3 μm particle size). The analytical conditions were based on those reported by Lyan *et al.*[40] with slight modifications: isocratic solvent system: methanol/TBME/water (700:280/20, v/v/v); flow rate = 0.4 mL/min, and detection wavelength at 298 nm. The identification of tocopherols in oils was ensured by comparing the retention times and absorption spectra of unknown peaks with those of reference standards and by adding α-, γ-, δ-tocopherol standards to the chromatographic sample. To generate the standard curves, five quantities of α-tocopherol (range: 0.1 - 2 μg), γ-tocopherol (range: 0.3 - 5 μg) and δ-tocopherol (range: 2 - 10 μg) were injected into the HPLC system (each standard being dissolved in 1 mL of the HPLC mobile phase: methanol/TBME/water (700:280:20, v/v/v)); the linear regression equation of each standard curve was then obtained by plotting the quantity of the standard compound injected against the peak surface area. The regression equation and correlation coefficient (R^2) were computed using ChromNav software (Thermo Scientific).

2.4 Sterols analysis by GC-FID

Unsaponifiable matter was determined using a modified method of Mounts[41]. Unsaponifiable fraction was determined by saponifying 50 mg of each oil with 5 mL methanolic potassium hydroxide (1 N) solution and 22 μL of 5 α-cholestanol solution (1 mg/mL) (Sigma-Aldrich) used as an internal standard in a capped flask for 16 h at room temperature. Ten milliliters of distilled water and 10 mL of dichloromethane were then added and mixed. The resulting solution was centrifuged and the organic fraction was kept in a flask. It was washed twice with 5 mL of water to neutralize the pH and stored. The solvent was subsequently evaporated to dryness under nitrogen at 40℃. After vortexing, the aliquot was derivatized to trimethylsilyl ether sterols (TMS) by the addition of 300 μL of NO-bis (trimethylsilyl) trifluoroacetamide and 50 μL of pyridine at 60℃ for 30 min, and then injected into the gas chromatograph (GC).

Samples were analyzed by GC with a Hewlett-Packard HP-4890D chromatograph equipped with a DB5 MS fused-silica capillary column (30 m, 0.25 mm i.d, 0.25 μm film thickness). The oven temperature was raised from 50 to 290℃ at a rate of 20℃/min. The flame ionization detector (FID) temperature was held at 290℃. The split ratio was 1:20. Hydrogen was used as the carrier gas at a pressure of 60 kPa. TMS were eluted from the column. The data were analyzed using EZChrom Elite software (Agilent Technologies, Massy, France). The areas of sterols were compared with the areas of known quantities of the internal standard (5 α-cholestane).

2.5 Polyphenol analysis

Three g of each oil was diluted in 6 mL petroleum ether and then cleansed with a silica solid phase extraction (SPE) cartridge conditioned by 6 mL of petroleum ether. The cartridge was then cleaned with 12 mL petroleum ether and dried for 10 minutes under nitrogen flux. Polyphenolic compounds were eluted with an 80/20 (v/v) mixture of methanol and distillated water, followed by 8 mL of acetonitrile. Under lowered pressure and at 50℃, the eluate was evaporated. The residue was dissolved in 1 mL of a 60/40 (v/v) combination of methanol and distilled water. A 0.45 μm nylon membrane was used to filter the obtained extract. Polyphenol analysis was performed with reverse phase (RP)/ HPLC. The HPLC experiments were carried out using a Perkin Elmer 200 system consisting of a degasser, a quaternary pump, an autosampler, and a diode array detection (DAD). Chromatographic separation was carried out on a Lichrospher 100 RP-18 column (150 mm × 4.6 mm, 5 μm). The elution conditions were as follows: mobile phase A (distilled water adjusted to pH 2.2 with trifluoroacetic acid) and mobile phase B (100% acetonitrile), with a flow rate of 1 mL/min, and an operating temperature of 27℃. The sample injected volume was 10 μL of seed oil sample and 900 μL of ethyl acetate. The running gradient was as follows: 0-5 min, 100% A; 5-50 min 40-100% B. The identification was particularly reached on the basis of spectroscopic data (UV-Vis) and retention time which were obtained from authentic standards. The measurement was achieved by using the DAD chromatograms set at 280 nm and the external calibration graph of a Quercetin standard. All compounds were later expressed as mg equivalents Quercetin per 100 g of oil. Lara-Spiral laboratory conducted the polyphenol analysis.

2.6 The 'Kit Radicaux Libres' test

The Kit Radicaux Libres (KRL) test was used to assess the oils overall antioxidant activity by their ability to protect erythrocytes against a controlled free radical attack at varying concentrations[10].

Diluted control blood samples were exposed to organic free radicals generated at 37℃ from the thermal decomposition of a solution of 2.2′–azobis (2-amidinopropane) dihy-

drochloride (AAPH). In order to record haemolysis, the turbidimetric optical density decline at 620 nm was measured using a 96-well microplate reader (KRL Reader, Kirial International). The antioxidant activity of the tested oil samples was expressed in Trolox equivalents and gallic acid equivalent. Lara-Spiral laboratory performed the KRL test (Couternon, France).

2.7 Statistical analysis

The data are expressed as means ± standard deviations (SDs). Statistical analyses included one-way analysis of variance (ANOVA), followed by a Turkey test to compare means that showed significant variation ($p < 0.05$) using STATISTICA software (version 10). *P*-values less than or equal to 0.05 were considered statistically significant. Principal component analysis (PCA) was used to display the correlation between the various parameters and their relationship with the different oils using XLSTAT[42].

3 Results and Discussion

3.1 Fatty acid composition

Fatty acid profiles of oil samples are regarded as an essential indicator of their nutritional value which could modify their physico-chemical properties[10]. Table 2 presents the fatty acid composition of the seed oils of pumpkin, melon, black cumin and that of argan oil. All the oil samples exhibited high amounts of total unsaturated fatty acids (consisting primarily of linoleic then oleic acid). Their amounts were of 74.61%, 74.49%, and 74.93%, respectively in PSO1, PSO2, and PSO3. Such values were lower than those observed in PSO4 (80.96%), melon seed oil (MSO) (82.77%), black cumin seed oil (83.67%), and argan oil (AO) (80.86%).

It is noteworthy that MSO witnessed the highest linoleic acid content (58.35%) and the lowest oleic acid one (22.87%) unlike AO which exhibited the highest amount of oleic acid (47.32%) and the lowest one in term of linoleic acid (32.6%).

From a nutritional point of view, melon and black cumin seed oils seem to be good sources of essential fatty acids, especially linoleic acid (C18:2 n-6) as compared to the other oil samples. According to Oomah *et al.*[43], this fatty acid may have beneficial physiological effects in the prevention of both coronary heart disease and cancer.

Due to the beneficial fatty acid composition, all Mediterranean vegetable oils could gain special attention as being

Table 2 Fatty acid content (% total FA) of Pumpkin seed oils (PSOs), Melon seed oil (MSO), *Nigella sativa* seed oil (NSO), and Argan oil (AO) (a).

Fatty acids	PSO1	PSO2	PSO3	PSO4	MSO	NSO	AO
C14:0 (Myristic acid)	0.10 ± 0.00^{c}	0.11 ± 0.00^{c}	0.11 ± 0.00^{c}	0.11 ± 0.00^{c}	0.06 ± 0.00^{d}	0.16 ± 0.00^{a}	0.14 ± 0.00^{b}
C15:0 (Pentadecylic acid)	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.047 ± 0.00^{b}	0.03 ± 0.00^{c}	0.05 ± 0.00^{b}
C16:0 (Palmitic acid)	16.42 ± 0.11^{a}	16.34 ± 0.13^{b}	16.26 ± 0.2^{c}	12.1 ± 0.03^{f}	10.76 ± 0.16^{g}	12.28 ± 0.05^{e}	12.80 ± 0.03^{d}
C16:1 (n-9) (Palmitoleic acid)	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.01 ± 0.00^{b}
C16:1 (n-7) (*trans*-palmitoleic acid)	0.11 ± 0.00^{c}	0.11 ± 0.00^{c}	0.11 ± 0.00^{c}	0.14 ± 0.00^{b}	0.11 ± 0.00^{c}	0.22 ± 0.00^{a}	0.11 ± 0.00^{c}
C17:0 (Margaric acid)	0.07 ± 0.00^{b}	0.08 ± 0.00^{a}	0.07 ± 0.00^{b}	0.06 ± 0.00^{c}	0.087 ± 0.00^{a}	0.07 ± 0.00^{b}	0.07 ± 0.00^{b}
C18:0 (Stearic acid)	8.06 ± 0.01^{a}	8.20 ± 0.02^{a}	7.93 ± 0.03^{ab}	6.10 ± 0.01^{b}	5.94 ± 0.02^{c}	3.47 ± 0.00^{e}	5.55 ± 0.02^{d}
C18:1 *trans* (Elaidic acid)	0.04 ± 0.02^{e}	0.07 ± 0.03^{d}	0.08 ± 0.00^{c}	0.01 ± 0.00^{f}	0.07 ± 0.03^{d}	0.10 ± 0.00^{b}	0.14 ± 0.02^{a}
C18:1 (n-9) (Oleic acid)	28.49 ± 0.03^{c}	27.47 ± 0.06^{d}	28.73 ± 0.05^{c}	34.12 ± 0.02^{b}	22.87 ± 0.08^{f}	24.68 ± 0.05^{e}	47.32 ± 0.02^{a}
C18:1 (n-7) (*trans*-vaccenic acid)	0.72 ± 0.01^{d}	0.70 ± 0.02^{d}	0.76 ± 0.01^{c}	0.72 ± 0.01^{d}	1.03 ± 0.03^{a}	0.98 ± 0.02^{b}	0.25 ± 0.02^{e}
C18:2 (n-6) (Linoleic acid)	44.87 ± 0.14^{d}	45.84 ± 0.04^{c}	44.9 ± 0.11^{d}	45.57 ± 0.04^{c}	58.35 ± 0.26^{a}	54.48 ± 0.08^{b}	32.60 ± 0.01^{e}
C20:0 (Arachidic acid)	0.50 ± 0.01^{a}	0.53 ± 0.00^{a}	0.51 ± 0.01^{a}	0.42 ± 0.00^{b}	0.23 ± 0.00^{d}	0.23 ± 0.00^{d}	0.35 ± 0.01^{c}
C20:1 (n-9) (Gondoic acid)	0.11 ± 0.00^{d}	0.11 ± 0.00^{d}	0.15 ± 0.00^{c}	0.10 ± 0.00^{d}	0.11 ± 0.00^{d}	0.38 ± 0.00^{a}	0.35 ± 0.00^{b}
C18:3 (n-3) (Linolenic acid)	0.25 ± 0.00^{b}	0.17 ± 0.00^{c}	0.18 ± 0.00^{c}	0.28 ± 0.00^{a}	0.23 ± 0.00^{bc}	0.29 ± 0.00^{a}	0.09 ± 0.00^{d}
C20:1 (n-7) (Paullinic acid)	ND	ND	ND	ND	ND	0.02 ± 0.01	ND
C20:2 (n-6) (*cis* 11,14-eicosadienoic acid)	ND	ND	ND	ND	ND	2.29 ± 0.02	ND
C22:0 (Behenic acid)	0.12 ± 0.00^{b}	0.14 ± 0.00^{a}	0.14 ± 0.00^{a}	0.13 ± 0.00^{a}	0.04 ± 0.00^{c}	0.05 ± 0.00^{c}	0.13 ± 0.00^{a}
C22:1 (n-9) (Erucic acid)	ND	ND	ND	ND	ND	0.22 ± 0.00	ND
C24:0 (Lignoceric acid)	0.07 ± 0.00^{b}	0.08 ± 0.00^{a}	0.03 ± 0.00^{d}	0.09 ± 0.00^{a}	0.04 ± 0.00^{c}	0.03 ± 0.00^{d}	0.05 ± 0.00^{c}
SAFA	25.39 ± 0.14^{a}	25.5 ± 0.01^{a}	25.07 ± 0.16^{a}	19.03 ± 0.04^{b}	17.21 ± 0.17^{c}	16.33 ± 0.60^{d}	19.13 ± 0.04^{b}
MUFA	29.49 ± 0.02^{c}	28.48 ± 0.07^{d}	29.85 ± 0.07^{c}	35.11 ± 0.01^{b}	24.20 ± 0.09^{f}	26.62 ± 0.05^{e}	48.17 ± 0.05^{a}
PUFA	45.12 ± 0.14^{cd}	46.01 ± 0.04^{c}	45.08 ± 0.11^{cd}	45.85 ± 0.04^{c}	58.57 ± 0.26^{a}	57.05 ± 0.08^{b}	32.69 ± 0.01^{d}

ND: Not Detected.

(a): SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Values are means ± SD of three independent determinations. Mean values with different letters in the same raw are significantly different.

PSO1: *Cucurbita maxima* seed oil obtained by MUV 2 65 Press; PSO2: *Cucurbita maxima* seed oil obtained by Komet DD 85 G Press; PSO3: *Cucurbita pepo* seed oil obtained by Komet DD 85 G Press; PSO4: *Cucurbita pepo* Subsp. *Pepo* purchased from Biocoop society; MSO: *Cucumis melo* seed oil obtained by Komet DD 85 G Press; NSO: *Nigella sativa* seed oil obtained by Komet DD 85 G Press; AO: Argan oil purchased from Biocoop society.

health-promoting. At the exception of AO, the low contents of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) and the high content of polyunsaturated fatty acids, made these unconventional oil sources interesting as potential dietary supplement[44].

It is worthy to point out that oleic acid (OA) is a bioactive nutrient known to cross the blood brain barrier. Hence, it prevents and/or treats neurodegenerative diseases whose frequency increases with age such as Alzheimer's and Parkinson's disease[45, 46]. This finding was consolidated by the research conducted by Debbabi *et al.*[47] on the protective effect of OA, on 7-ketocholesterol (7KC)-induced mitochondrial and peroxisomal dysfunction in murine microglial BV2 cells. Such a lipid peroxidation product (7KC) may be increased in the body fluids and tissues of patients with age related diseases, including neurodegenerative diseases, and trigger microglial dysfunction involved in neurodegeneration[6, 48]. Debbabi *et al.*[47] proved that OA was able to impair 7KC-induced loss of mitochondrial transmembrane potential ($\Delta\Psi m$), which is associated with increased permeability to propidium iodide, an indicator of cell death. Furthermore, in recent studies, Ubaid *et al.*[49] investigated the impact of combining OA with camel α-lactalbumin (α-LA) in preventing Parkinson's disease and validated the neuroprotective role of the complex which was more effective than the individual entity, i.e., α-LA or OA.

In term of saturated fatty acids, palmitic acid (C16:0) is the most abundant in oils, followed by stearic acid (C18:0). PSO1 showed the highest amounts in term of these two fatty acids (16.42% for palmitic acid *vs* 8.06% for stearic acid). Very long-chain saturated fatty acids (C22:0 and C24:0) were found in all oil samples (range from 0.04% to 0.14% for C22:0 and from 0.03% to 0.09% for C24:0). *Trans* C18:0 n-9 (elaidic acid) content was low and significantly ($p<0.05$) different in all oil samples (range from 0.01% in PSO4 to 0.14% in AO). These results were confirmed by the findings of Rezig *et al.*[23] for *Cucurbita maxima*, of Rezig *et al.*[21] for *Cucurbita pepo* L. and *Cucumis melo* L., of Fruhwirth & Hermetter[50] for *Cucurbita pepo* subsp *pepo* var. Styriaca, and of Zarrouk *et al.*[10] for black cumin and argan oils.

For NSO, C20:0 n-6 (arachidic acid) content was of 2.29%. This saturated fatty acid was previously reported by Zarrouk *et al.*[10] for Tunisian black cumin origin but not in Moroccan and Egyptian ones[51]. In accordance with other studies conducted by Zarrouk *et al.*[10] and Gharby *et al.*[52], we observed the presence of traces of fatty acids in NSO such as eicosenoic acid (C20:0 n-9, 0.38%) and erucic acid (C22:0 n-9, 0.22%). These results were in compliance with previously published data, showing oleic acid (41.2% - 45.75%) as the major unsaturated fatty acid detected, followed closely by linoleic acid (31.99% - 37.9%) and palmitic acid (12.11% - 16.5%)[10, 53, 54].

3.2 Tocopherols

Tocopherol composition is regarded as an essential attribute in identifying the potential benefits of vegetable oils for protection against oxidative stress. Tocopherol composition of the oil samples investigated in this study is given in Table 3. Among the tocopherol content, a wide variation, and significant differences ($p<0.05$) were observed. Melon seed oil (MSO) had significantly higher total tocopherol content ($p<0.05$) followed in descending order by AO, PSO4, PSO2, PSO3, and NSO. At the exception of NSO, in which δ-tocopherol was the only tocopherol detected, the γ-tocopherol was present in a higher level in all oil samples

Table 3 Tocopherol content (mg/kg) of Pumpkin seed oils (PSOs), Melon seed oil (MSO), *Nigella sativa* seed oil (NSO), and Argan oil (AO) (a).

Oils	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Tocopherol content
PSO1	ND	226.03 ± 22.72^{f}	67.77 ± 2.78^{d}	293.80 ± 25.27^{e}
PSO2	ND	272.70 ± 8.29^{d}	91.00 ± 4.55^{c}	363.70 ± 5.01^{d}
PSO3	ND	245.70 ± 10.44^{e}	49.17 ± 3.60^{e}	294.87 ± 13.91^{e}
PSO4	28.85 ± 1.49^{b}	326.10 ± 2.83^{c}	42.3 ± 1.70^{f}	397.25 ± 3.04^{c}
MSO	ND	633.1 ± 18.81^{a}	19 ± 0.90^{g}	652.1 ± 3.26^{a}
NSO	ND	ND	118.85 ± 12.23^{a}	118.85 ± 12.23^{f}
AO	37.3 ± 0.14^{a}	466.85 ± 0.64^{b}	99.85 ± 4.45^{b}	604.4 ± 5.23^{b}

ND: Not Detected.

(a) Values are means ± SD of three independent determinations. Mean values with different letters in the same column are significantly different.

PSO1: *Cucurbita maxima* seed oil obtained by MUV 2 65 Press; PSO2: *Cucurbita maxima* seed oil obtained by Komet DD 85 G Press; PSO3: *Cucurbita pepo* seed oil obtained by Komet DD 85 G Press; PSO4: *Cucurbita pepo* Subsp. *Pepo* purchased from Biocoop society; MSO: *Cucumis melo* seed oil obtained by Komet DD 85 G Press; NSO: *Nigella sativa* seed oil obtained by Komet DD 85 G Press; AO: Argan oil purchased from Biocoop society.

(range from 76.93% for PSO1 to 97.08% for MSO). In addition, α-tocopherol was present only in PSO4 and AO with respective contents of 7.26% and 6.17%. According to Fatnassi *et al.*[55], α-tocopherol is recommended for human and animal consumption due to its higher biological activity when compared to other tocopherols whilst γ-tocopherol may be a more potent antioxidant than α-tocopherol[56]. For comparison reason, the total tocopherol content of MSO was higher than that found by Mallek-Ayadi *et al.*[28] in *Cucumis melo* L. Maazoun variety (270.7 mg/kg) and by da Silva & Jorge[57] in *inodorus* Naudin melon seed oil (270.2 mg/kg). AO was reported for its high content of tocopherols, with γ-tocopherol being the major one[53]. The total AO tocopherol content found in our study was higher than that found by Zarrouk *et al.*[10] either in unroasted-cosmetic (Berkane) (384 mg/kg), or in roasted-dietary AO originated from Agadir (453 mg/kg) and Berkane (458 mg/kg). It is worth noting, that at the exception of α-tocopherol which witnessed a higher level in roasted-dietary AO originated from Agadir (429 mg/kg), all tocopherol isoform contents were lower than those observed in our study.

The total tocopherol content of PSO2 was lower than that found by Rezig *et al.*[23] on *Cucurbita maxima* seed oil extracted by a Komet DD-85G Press (599.33 mg/kg). For *Cucurbita pepo* seed oil, the total tocopherol contents observed in PSO3 and PSO4 were lower than that cited by Nyam *et al.*[58] (806.5 mg/kg). The γ-tocopherol content was even lower (76.03%) than those found in our study (83.32% for PSO3 and 82.08% for PSO4). Furthermore, total tocopherol content in NSO was in compliance with those reported by Matthaus & Özcan[59] in 13 *Nigella sativa* seed oil samples ranging from 19 mg/kg and 155.9 mg/kg. Unlike our study in which only the γ-tocopherol was detected, all tocopherol isoforms were present with a wide variation between all seed oil samples. According to Nawirska-Olszańska *et al.*[60], the genetic factors, extraction technique, cultivation, climate, production and storage conditions of seed oils are critical factors for the content of bioactive compounds.

Numerous compounds present in the Mediterranean diet such as tocopherol isoforms have been shown to be able to counteract the toxic effects of cytotoxic oxysterols such as 7KC, 7β-hydroxycholesterol and 24S-hydroxycholesterol, and to prevent 7KC-associated diseases, including neurodegenerative diseases[5, 61]. Thus, γ-tocopherol, which is at elevated levels in all Mediterranean vegetable oils studied and which can cross the blood brain barrier, could be useful to attenuate the toxic effects of 7KC. Such hypothesis was confirmed by Debbabi *et al.*[47] who showed that γ-tocopherol was able to impair 7KC-induced loss mitochondrial transmembrane potential, which is associated with increased permeability to propidium iodide, an indicator of plasma membrane damages and murine microglial BV-2 cell death. This isomer was also able to prevent the decrease in ABCD3 protein levels, which allows the evaluation of peroxisomal mass, and in mRNA levels of ABCD1 and ABCD2, which encode for two peroxisomal transporters involved in peroxisomal β-oxidation.

Zakharova *et al.*[62] also investigated the efficacy of combining insulin and α-tocopherol to increase the viability of cultured cortical neurons under oxidative stress conditions and to normalize metabolic disturbances caused by free radical reaction activation in rats with two-vessel forebrain ischemia/reperfusion injury in the brain cortex. The latter demonstrated that α-tocopherol improved the protective and antioxidative actions of insulin on neurons. On the other hand, Mitra *et al.*[63] proved that short term alpha-tocopherol therapy showed improvement in motor dysfunction and reduction of demyelination in the female Sprague-Dawley rats model of multiple sclerosis which is defined as an autoimmune disorder characterized by demyelination and axonal loss.

The latter findings could support the involvement of tocopherol isomers which can act on the function of organelles involved either in age-related diseases, particularly in neurodegenerative diseases or in other diseases such as multiple sclerosis.

3.3 Phytosterols

Phytosterols belong to the unsaponifiable lipid fraction of vegetable oils and are considered to possess health benefits. In fact, phytosterols have an effect on the human body by inhibiting cholesterol absorption from the intestine and decreasing blood levels of low density lipoprotein cholesterol fraction (LDLc)[64]. Indeed, sitosterol contributes to lower blood LDL cholesterol by 10 - 15% as part of a healthy diet[65]. Phytosterol compounds identified in oil samples are illustrated in Table 4. It is interesting to mention that 10 phytosterol compounds were identified either in *Cucurbita maxima* seed oil (PSO1, PSO2) or in *Cucurbita pepo* L. seed oil (PSO3). Among these phytosterols, spinasterol was predominant (ranging from 1514.58 μg/g for PSO2 to 1609.46 μg/g for PSO1) followed by 5,24-stigmastadienol (ranging from 1360.75 μg/g for PSO2 to 1509.57 μg/g for PSO3). At the exception of brassicasterol, campestanol, stigmasterol, and Δ7-campesterol, PSO4 exhibited an identical phytosterol feature than those found in PSO1, PSO2, and PSO3. In fact, spinasterol and 5,24-stigmastadienol were predominant at respective levels of 1598.38 μg/g and 731.43 μg/g.

It is worth noting that 5,24-stigmastadienol, which was the only phytosterol compound identified in MSO, was more abundant (1633.51 μg/g) than those found in *Cucurbita maxima* and *Cucurbita pepo* L. seed oil samples.

For NSO, 12 phytosterol compounds were identified with a relatively high level in cholesterol (17.46 μg/g) when compared to the oil samples concerned by this study. Sitosterol was the most abundant phytosterol (1071.19 μg/g) followed

Table 4 Sterol content (μg/g) of Pumpkin seed oils (PSOs), Melon seed oil (MSO), *Nigella sativa* seed oil (NSO), and Argan oil (AO) (a).

Sterols	PSO1	PSO2	PSO3	PSO4	MSO	NSO	AO
Cholesterol	15.79 ± 4.70^{b}	8.05 ± 0.54^{d}	11.43 ± 0.63^{c}	6.88 ± 1.45^{e}	ND	17.46 ± 1.87^{a}	ND
Brassicasterol	47.04 ± 1.33^{b}	53.25 ± 0.47^{a}	52.21 ± 1.48^{a}	ND	ND	ND	ND
Campesterol	78.33 ± 1.36^{c}	69.65 ± 1.55^{d}	84.94 ± 2.22^{b}	54.79 ± 3.19^{e}	ND	195.18 ± 12.31^{a}	ND
Campestanol	29.45 ± 1.45^{a}	26.09 ± 1.77^{b}	29.77 ± 1.26^{a}	ND	ND	19.98 ± 1.12^{c}	ND
Stigmasterol	124.93 ± 3.71^{c}	120.80 ± 2.70^{d}	139.66 ± 3.86^{b}	ND	ND	225.79 ± 15.00^{a}	ND
Δ7-Campesterol	88.02 ± 2.29^{c}	105.18 ± 2.22^{b}	175.53 ± 4.85^{a}	ND	ND	ND	32.69 ± 1.03^{d}
Spinasterol	1609.46 ± 30^{a}	1514.58 ± 42.08^{d}	1577.12 ± 47.07^{c}	1598.38 ± 127.45^{b}	ND	ND	592.05 ± 6.11^{e}
5,24-Stigmastadienol	1436.78 ± 23.48^{c}	1360.75 ± 36.26^{d}	1509.57 ± 48.48^{b}	731.43 ± 58.84^{e}	1633.51 ± 55.08^{a}	ND	ND
Δ7-Stigmastenol	493.24 ± 18.75^{c}	441.55 ± 8.15^{d}	508.42 ± 14.85^{b}	217.29 ± 16.71^{e}	ND	ND	673.20 ± 6.04^{a}
Δ7-Stigmasterol	ND	ND	ND	ND	ND	ND	46.24 ± 0.25
Δ7-avenasterol	695.48 ± 17.57^{a}	667.05 ± 21.67^{b}	647.33 ± 21.31^{c}	545.11 ± 60.26^{d}	ND	ND	82.54 ± 1.17^{e}
Clerosterol	ND	ND	ND	ND	ND	31.96 ± 4.79	ND
Sitosterol	ND	ND	ND	ND	ND	1071.19 ± 66.84	ND
Δ5-Avenasterol	ND	ND	ND	ND	ND	231.53 ± 10.07	ND
β-amyrine	ND	ND	ND	ND	ND	76.58 ± 3.85^{ab}	76.72 ± 10.28^{a}
Graminasterol	ND	ND	ND	ND	ND	58.53 ± 3.77	ND
Cycloartenol	ND	ND	ND	ND	ND	631.51 ± 39.40^{a}	184.92 ± 17.78^{b}
24 methylene cycloartenol	ND	ND	ND	ND	ND	400.40 ± 37.5^{a}	46.94 ± 2.44^{b}
Citrostadienol	ND	ND	ND	ND	ND	102.02 ± 16.79^{a}	69.63 ± 4.15^{b}
Unknown	327.64 ± 24.68^{d}	284.33 ± 10.24^{e}	268.23 ± 12.43^{f}	573.94 ± 104.67^{a}	5332.03 ± 261.05^{b}	253.46 ± 28.06^{g}	432.05 ± 30.44^{c}
Total content (μg/g)	4946.12 ± 110.17^{c}	4651.30 ± 109.23^{d}	5004.23 ± 147.41^{b}	3727.81 ± 289.84^{e}	6995.55 ± 224.01^{a}	3315.61 ± 196.27^{f}	2237.00 ± 37.55^{g}

ND: Not Detected.

(a) Values are means ± SD of three independent determinations. Mean values with different letters in the same raw are significantly different.

PSO1: *Cucurbita maxima* seed oil obtained by MUV 2 65 Press; PSO2: *Cucurbita maxima* seed oil obtained by Komet DD 85 G Press; PSO3: *Cucurbita pepo* seed oil obtained by Komet DD 85 G Press; PSO4: *Cucurbita pepo* Subsp. *Pepo* purchased from Biocoop society; MSO: *Cucumis melo* seed oil obtained by Komet DD 85 G Press; NSO: *Nigella sativa* seed oil obtained by Komet DD 85 G Press; AO: Argan oil purchased from Biocoop society.

by cycloartenol (631.51 μg/g), and 24 methylene cycloartenol (400.40 μg/g).

In AO, nine phytosterol compounds were identified among which Δ7-stigmastenol and spinasterol were present in higher levels (672.2 μg/g and 592.05 μg/g, respectively).

In term of richness in phytosterol content, MSO exhibited the highest content (6995.55 μg/g) followed in descending order by PSO3, PSO1, PSO2, PSO4, NSO, and AO.

For *Cucurbita maxima* seed oil, phytosterol composition was not in compliance with that cited by Rezig *et al.*[66] in *Cucurbita maxima* var. 'Béjaoui' seed oil extracted with petroleum ether. Cholesterol, stigmasterol, 5,24-stigmastadienol, and Δ7-avenasterol were the only common phytosterol compounds with a relatively high content in cholesterol (25.35 mg/100 g) and an abundance of sitosterol (50.64 mg/100 g). Phytosterol composition of *Cucurbita pepo* L. seed oil was not in accordance with those reported by Akin *et al.*[67] in four cold pressed *Cucurbita pepo* seed oil samples originated from the Central Anatolia region of Turkey. In fact, 5 phytosterols were identified (spinasterol, β-sitosterol, Δ7,22,25-stigmastatrienol, Δ7-stigmasterol) with a predominance of spinasterol+β-sitosterol.

Phytosterol composition of MSO was not in agreement with that cited by Mallek-Ayadi *et al.*[28] in 'Maazoun' *Cucumis melo* variety seed oil extracted by hexane. In fact, the latters found that β-sitosterol was the predominant phytosterol compound identified (206.42 mg/100 g) followed by Δ5,24-stigmastadienol (117.91 mg/100 g). It is worth mentioning that β-sitosterol, not identified in our study, was also present in melon seed oil (*inodorus* Naudin variety) and in *tibish* melon seed oil with respective contents of 210.2 md/100 g and 289 mg/100 g[57, 68].

Total phytosterol content of NSO was higher than that reported by Zarrouk *et al.*[10] with a closely phytosterol compound identification and a predominance of sitosterol (32.3%).

Even schotenol was not identified in our study in AO, the abundance of spinasterol (26.46%) was confirmed by previous studies conducted by Khallouki *et al.*[53], Jordan *et al.*[69], and Zarrouk *et al.*[10] with respective percentages of 40%, 38.98%, and a range from 33.22% to 36.42% for roasted-dietary argan oils originated from Berkane and Agadir.

In line with the effect of the intake of phytosterols on the development of neurodegenerative diseases, these bioactive nutrients have been recently reported to possess anti-neurodegenerative effects[70, 71] by treating neuroinflammation which is a common pathological feature of many neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease[72–74]. In fact, phytosterols act by lowering cholesterol levels in the brain. One study showed that phytosterol administration slightly reduced brain membrane cholesterol, interacted loosely with saturated phospholipids, which presumably reduced membrane mo-

lecular order and fluidity[75, 76]. It has also been reported that elevated levels of plant sterols in the brain can moderately alter cholesterol metabolism in specific brain regions[77]. Supplementation with a phytosterol cocktail consisting of sitosterol (60%), campesterol (25%), and stigmasterol (15%) was also reported to suppress the expressions of pro-inflammatory factors, including tumor necrosis factor alpha (TNF-α), CCL2 (also known as monocyte chemoattractant protein-1, MCP-1), gamma interferon (IFN-γ), and interleukin-6 (IL-6), and to upregulate the expression of IL-10 (an anti-inflammatory cytokine) in experimental autoimmune encephalomyelitis[78, 79]. Besides, phytosterols were also reported to be readily available in the brain, as they can cross the blood brain barrier and have a significant impact on neuronal development[80–82]. In an *in vivo* study, Saeed *et al.*[83] reported a significant elevation of plant sterol concentration in cerebrum following the administration of dietary phytosterols and supposed that transmission across blood brain barrier might be facilitated by endothelial cells and astrocytes[83].

Richness of all Mediterranean vegetable oil in phytosterols could be an alternative in preventing or treating the age-related diseases and particularly neurodegenerative ones by considering such oil sources as a potential dietary supplement.

3.4 Polyphenol content

Table 5 depicts the polyphenol composition of the seed oil samples. The cold pressed *Cucurbita maxima* var. 'Béjaoui' seed oil obtained by the MUV 2 65 Press (PSO1) exhibited the highest polyphenol content (13.17 mg equivalents quercetin/100 g) followed in descending order by black cumin seed oil (4.75 mg equivalents quercetin/100 g), melon seed oil (2.61 mg equivalents quercetin/100 g), *Cucurbita pepo* Subsp. *pepo* purchased from Biocoop Society (PSO4) (0.75 mg equivalents quercetin/100 g), cold pressed *Cucurbita maxima* var. 'Béjaoui' seed oil (PSO2) (0.7 mg equivalents quercetin/100 g), and argan oil (0.68 mg equivalents quercetin/100 g). The lowest polyphenol content (0.44 mg equivalents quercetin/100 g) was observed in the *Cucurbita pepo* seed oil var. 'Essahli' obtained by the Komet DD 85 G Press (PSO3).

Total phenolic content in PSO1 was higher than those previously reported by Rezig *et al.*[23] in pumpkin (*Cucurbita maxima*) seed oil of the 'Béjaoui' Tunisian cultivar extracted by hexane, pentane, chloroform/methanol, and by the Komet DD 85 G Press with respective contents of 23.32 ± 2.85 mg gallic acid equivalents (GAE)/kg, 23.27 ± 2.14 mg GAE/kg, 54.41 ± 4.12 mg GAE/kg, and 39.77 ± 2.53 mg GAE/kg. For comparison reasons, it is worth noting mentioning that literature related to total polyphenol content in cold pressed *Cucurbita pepo* and melon seed oils is scarce. Hashemi *et al.*[84] reported a total phenolic contents of 144 mg GAE/kg and 125 mg GAE/kg, respectively in pumpkin (*Cucurbita pepo* subsp *Pepo* var. Styriaca) and Mashhadi melon (*Cucumis melo* var. Iranians cv. Mashhadi) seed oils extracted by *n*-hexane. These values are higher than those obtained in PSO4 and in MSO.

The total phenolic content in AO was in the same range than that reported by Badreddine *et al.*[13] in Moroccan dietary argan oils originating from Agadir (0.54 mg equivalents quercetine (EQ)/100 g of oil) and Berkane (0.75 mg EQ/100 g of oil). Variances in total phenolic content recorded in each oil sample could be due to differences in extraction procedures, analytical methods, and environmental conditions of plant or harvesting time[23].

Phenolic compounds are responsible for some of the properties of oil, including bitterness and astringency. HPLC analysis of phenolic compounds of all pumpkin seed oils revealed the presence of only an unidentified peak at 13.3 min (data not shown). Such a result is in accordance with that reported by Rezig *et al.*[23] on cold *Cucurbita maxima* var. 'Béjaoui' seed oils and those extracted by hexane, pentane, and chloroform/methanol. In fact, the

Table 5 Polyphenol content (mg equivalents Quercetin/100 g) of Pumpkin seed oils (PSOs), Melon seed oil (MSO), *Nigella sativa* seed oil (NSO), and Argan oil (AO).

Polyphenols	PSO1	PSO2	PSO3	PSO4	MSO	NSO	AO
Vanillin	ND	ND	ND	ND	0.59	ND	ND
p-hydroxycinnamic acid	ND	ND	ND	ND	ND	0.04	ND
Coumarine	ND	ND	ND	ND	ND	0.05	ND
thymoquinone	ND	ND	ND	ND	ND	1.2	ND
Sum of identified pics	–	–	–	–	0.59	1.29	–
Sum of 280 nm pics	13.17	0.7	0.44	0.75	2.61	4.75	0.68

ND: Not Detected. PSO1: *Cucurbita maxima* seed oil obtained by MUV 2 65 Press; PSO2: *Cucurbita maxima* seed oil obtained by Komet DD 85 G Press; PSO3: *Cucurbita pepo* seed oil obtained by Komet DD 85 G Press; PSO4: *Cucurbita pepo* Subsp. *Pepo* purchased from Biocoop society; MSO: *Cucumis melo* seed oil obtained by Komet DD 85 G Press; NSO: *Nigella sativa* seed oil obtained by Komet DD 85 G Press; AO: Argan oil purchased from Biocoop society.

HPLC-DAD-ESI-MS/MS analysis proved the presence of an unidentified peak at a retention time of 13.18 min with a *m/z* $(M-H)^-$ of 429. Subjected to MS^n analysis, the *m/z* 429 produced signals at *m/z* 277 and *m/z* 266. The peak was hypothesized to correlate to two typical phenolic acid derivatives of the pumpkin seed. At the exception of the unidentified peak, these authors identified *p*-hydroxybenzoic and *p*-hydroxybenzaldehyde. The respective contents varied between 0.11 ± 0.01 mg/kg for cold pressed *Cucurbita maxima* seed oil and 23.78 ± 2.54 mg/kg for that extracted by chloroform/methanol and 0.59 ± 0.06 mg/kg for *Cucurbita maxima* seed oil extracted by pentane and 138.46 ± 12.59 mg/kg for that extracted by chloroform/methanol. Moreover, in cold pressed *Cucurbita pepo* var. 'Essahli' seed oil, Rezig *et al.*[21] identified two phenolic classes by HPLC analysis namely phenolic acids (caffeic and vanillic) and lignan (pinoresinol). Pinoresinol was the major phenolic compound (7.92 ± 0.81 μg/g) followed by caffeic and vanillic acids which were present in an equal amount of 2.33 μg/g. In terms of phenolic compounds in MSO, only vanillin was identified (0.59 mg equivalents quercetin/100 g). This compound was also identified by Mohammad *et al.*[85] in two cultivars of winter melon seed oils (4.78 ± 1.8 μg/g for cultivar 1 and 8.86 ± 2.32 μg/g for cultivar 2). The authors also identified gallic acid, 3,4-dihydroxybenzoic acid, 3,4-dihydrobenzaldehyde, vanillic acid, *p*-coumaric acid, *trans*-cinnamic acid, and ferulic acid. Moreover, our result is not in agreement with the finding of Mallek-Ayadi *et al.*[28] in 'Maazoun' variety *Cucumis melo* seed oil extracted by hexane who identified phenolic acids, flavonoids, secoiridois, and lignans. In terms of phenolic acids, gallic acid was predominant (7.26 ± 0.02 μg/g), followed in descending order by caffeic acid (3.13 ± 0.00 μg/g), rosmarinic acid (2.91 ± 0.04 μg/g), and protocatechuic acid (0.89 ± 0.01 μg/g). Amentoflavone was the predominant compound in flavonoids (32.8 ± 0.21 μg/g) followed by luteolin-7-O-glycoside (9.6 ± 0.01 μg/g), naringenin (4.72 ± 0.01 μg/g), apigenin (3.88 ± 0.03 μg/g) and flavone (1.94 ± 0.02 μg/g). Oleuropein (1.65 ± 0.07 μg/g) and pinoresinol (3.95 ± 0.03 μg/g) were the main compounds in the secoiridois and lignans phenolic classes, respectively. For AO, no phenolic acid was identified by HPLC analysis. Such a result is not in agreement with those previously cited by Zarrouk *et al.*[10] and Badreddine *et al.*[13]. In fact, tyrosol was the only phenolic compound identified by Badreddine *et al.*[13] in Moroccan dietary argan oils originating from Agadir (0.07 mg equivalents quercetin/100 g) and Berkane (0.06 mg equivalents quercetine/100 g). For *Nigella sativa* seed oil, the HPLC analysis revealed the presence of *p*-hydroxycinnamic acid (0.04 mg equivalents quercetin/100 g), coumarine (0.05 mg EQ/100 g) and thymoquinone (1.2 mg EQ/100 g). The amount of thymoquinone was higher than that reported by Meddeb *et al.*[86] on *Nigella sativa* seed oil (0.7 mg EQ/100 g). Other phenolic compounds were also identified such as homovanillic acid (0.19 mg EQ/100 g), vanillin (0.23 mg EQ/100 g), quercetin (0.13 mg EQ/100 g), 2,6-dihydroxybenzoic acid (1.27 mg EQ/100 g), chlorogenic acid (0.09 mg EQ/100 g), and ferulic acid (0.09 mg EQ/100 g).

Dugas *et al.*[87] and Cilla *et al.*[88] reported the efficiency of phenolic compounds such as resveratrol, quercetin, epigallocatechin-3-gallate, catechin, epicatechin, tyrosol, hydroxytyrosol, rutin, and kaempferol in reducing the 7KC-induced side effects involved in age related macular degeneration and neurodegenerative diseases. Because of the nature of these natural molecules, often associated with the Mediterranean diet, it may be thought that regular intake of these nutrients, particularly through the diet, could reduce the toxicity of 7KC and the development of 7KC-associated diseases.

3.5 "Kit Radicaux Libres" test

The antioxidant activities of the different oils, evaluated by the KRL test, were illustrated as mean ± SD in Table 6. The antioxidant activities of the studied oils were expressed in Trolox and gallic acid equivalent (mole Trolox/mL of oil; mole of gallic acid/mL of oil). Based on the Turkey test, the antioxidant properties of the oils permit to distinguish two groups of oils and are in the following order: NSO > (PSO1, PSO2, PSO3, PSO4, MSO, AO).

To the best of our knowledge, and for comparison reasons, it is worth noting that no previous studies were previously recorded on the antioxidant activities of Cucurbitaceae seed oils by the KRL *in vitro* assay. However, in another study conducted by Zarrouk *et al.*[10], the antioxidant activities of roasted argan oils originating from Agadir and Berkane were higher than that observed in our study (6372 ± 318 Trolox equivalents *vs* 7324 ± 376 Trolox equivalents). Variances in the antioxidant activities of AO could be attributed to the roasting process parameters (temperature, time) which enhances the erythrocytes protection against the free radical attack. This hypothesis was consolidated by the findings of Belcadi-Haloui *et al.*[89]. Indeed, these researchers showed that the oxidative stability of argan (*Argania spinosa* L.) oil was improved after roasting at 175℃/30 min. Such a result was due to the increase in phospholipid content and the formation of Maillard reaction products which are endowed with antioxidant activity. The higher antioxidant activity of NSO, compared to the other studied oils, could be attributed to its richness in thymoquinone (1.2 mg EQ/100 g). In fact, this chemical component is recognized as the main bioactive compound of the *Nigella sativa* endowed with a strong antioxidant activity[90].

3.6 Chemometric analysis

The phytochemical content and antioxidant activity assessed using the KRL *in vitro* assay of the different

Table 6 Kit Radicaux Libres (KRL) assay for estimating antioxidant activity of Pumpkin seed oils (PSOs), Melon seed oil (MSO), *Nigella sativa* seed oil (NSO), and Argan oil (AO) (a).

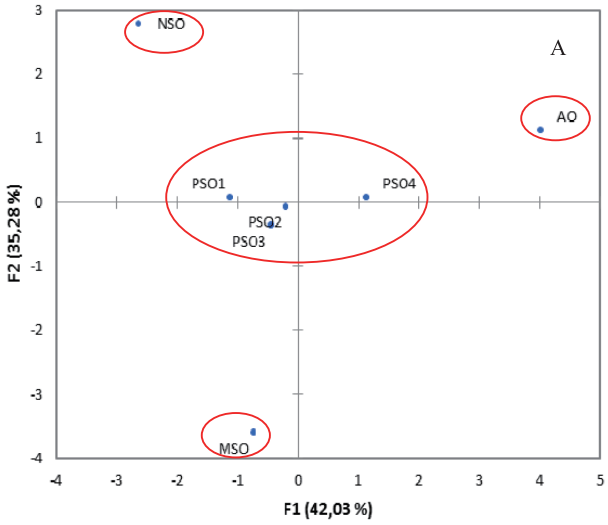
Oils	KRL Test (Trolox Equivalent)	KRL Test (Gallic Acid Equivalent)
Control	0	0
PSO1	9.32 ± 4.77^{b}	4.74 ± 2.3^{b}
PSO2	6.39 ± 0.75^{b}	3.3 ± 0.82^{b}
PSO3	10.94 ± 6.29^{b}	5.35 ± 2.63^{b}
PSO4	14.57 ± 4.67^{b}	7.25 ± 1.58^{b}
MSO	11.35 ± 6.45^{b}	5.55 ± 2.69^{b}
NSO	31.78 ± 5.94^{a}	15.99 ± 1.24^{a}
AO	9.02 ± 3.84^{b}	4.56 ± 1.77^{b}

(a) Data are presented in Trolox and gallic acid Equivalents: 1 mL of oil is equivalent to X mole (values shown in the Table) of Trolox or gallic acid. Data shown are mean of three independent experiments realized in triplicate. Data shown are the mean ± SD of three independent experiments conducted in triplicate. Superscript letters in the same column indicate significant difference between values at $p < 0.05$ level.

PSO1: *Cucurbita maxima* seed oil obtained by MUV 2 65 Press; PSO2: *Cucurbita maxima* seed oil obtained by Komet DD 85 G Press; PSO3: *Cucurbita pepo* seed oil obtained by Komet DD 85 G Press; PSO4: *Cucurbita pepo* Subsp. *Pepo* purchased from Biocoop society; MSO: *Cucumis melo* seed oil obtained by Komet DD 85 G Press; NSO: *Nigella sativa* seed oil obtained by Komet DD 85 G Press; AO: Argan oil purchased from Biocoop society.

studied oils were compared using principal component analysis (PCA). PCA provides a graphical representation of inter-sample (scores-plot) (Fig. 1A) and inter-variable (loading-plot) (Fig. 1B) relationships and provides a way to reduce the complexity of the data. The first (F1) and the second (F2) principal components were sufficient to display the structure of the data, since they explained 73.31% of the total variance.

By examining the scores-plot in the area defined by F1 and F2, it is interesting to mention that the studied parameters can be used as a tool to discriminate the studied oils, and permitted to separate the samples into four groups, based on their minor contents. Group I, located at the top of the scores-plot, and correlated positively to F2 and negatively to F1, consisted of the NSO. Such group was characterized by the highest levels of KRL and δ-tocopherol, and the lowest levels of total tocopherol content (TTC). Group II, located on the right-side of the scores-plot, and correlated positively to F1 and F2, included AO. Such group was characterized by having the highest content of α-tocopherol and MUFA. Group III, located on the bottom of the scores-plot, and correlated negatively to F1, and F2, included MSO. Such group was characterized by having the highest content of γ-tocopherol and TTC. Group IV, located on the center of the scores-plot, included PSO1, PSO2,



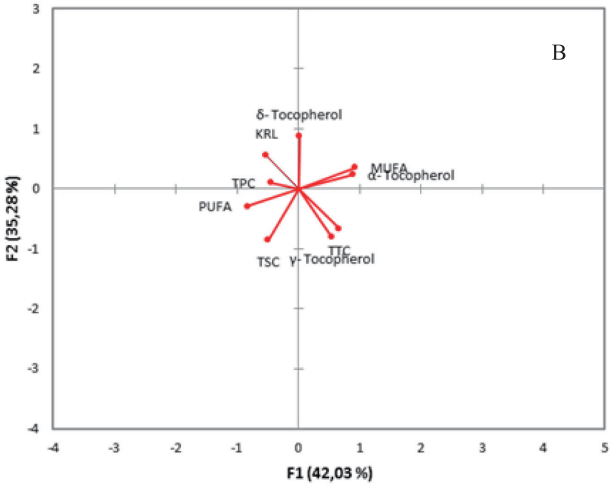


Fig 1 Principal Component Analysis (scores (A) and loading plots (B), biplot) applied to dataset of different phytochemical compounds concentration (MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; TTC: Total Tocopherol Content; TPC: Total Polyphenol Content; TSC: Total Sterol Content) and total antioxidant activity (KRL: Kit Radicaux Libres). PSO1: *Cucurbita maxima* seed oil obtained by MUV 2 65 Press; PSO2: *Cucurbita maxima* seed oil obtained by Komet DD 85 G Press; PSO3: *Cucurbita pepo* seed oil obtained by Komet DD 85 G Press; PSO4: *Cucurbita pepo* Subsp. *Pepo* purchased from Biocoop society; MSO: *Cucumis melo* seed oil obtained by Komet DD 85 G Press; NSO: *Nigella sativa* seed oil obtained by Komet DD 85 G Press; AO: Argan oil purchased from Biocoop society.

PSO3 and PSO4. PSO1, PSO2 and PSO3 were located negatively to F1 and F2. They are characterized by a high level of total sterol content (TSC) and no detection of α-tocopherol. PSO4 was correlated positively to F1 and F2 and was characterized by the high level of α-tocopherol. Therefore, the results obtained from the chemometric evaluation of the minor bioactive compounds and antioxidant activities of the several Mediterranean vegetable oils lead to conclude that these data could be used as an alternative way for the discrimination between the different Mediterranean vegetable oils.

4 Conclusion

The present study was undertaken to establish the fatty acid profile, the tocopherol, the phytosterol, and the polyphenol profiles of pumpkin, melon, black cumin seed oils and that of dietary argan oil. The KRL test was also used to study the antioxidant activities of the Mediterranean oil seeds concerned by this investigation. On the basis of the obtained results, cold extracted melon seed oil (MSO) exhibited a typical bioactive profile with the highest content in polyunsaturated fatty acids (58.57 ± 0.26%), tocopherols (652.1 ± 3.26 mg/kg), and phytosterols (6999.55 ± 224.01 μg/g). HPLC analysis revealed that pumpkin (*Cucurbita maxima* var. 'Béjaoui') seed oil extracted by the MUV2 65 screw press (Smir Technotour, Agadir, Morocco) named as PSO1 exhibited the highest content in phenolics (13.17 mg EQ/100 g). When compared to the other oils, *Nigella sativa* seed oil (NSO) was endowed with a strong antioxidant activity (31.78 ± 5.94 Trolox Equivalent). Such activity could be attributed to the presence of thymoquinone (1.2 mg EQ/100 g), recognized as a powerful antioxidant bioactive compound. The phytochemical content and antioxidant activity assessed using the KRL *in vitro* assay of the different studied oils were compared using principal component analysis (PCA). The first (F1) and the second (F2) principal components were sufficient to display the structure of the data, since they explained 73.31% of the total variance. By examining the scores-plot in the area defined by F1 and F2, it is interesting to mention that the studied parameters can be used as a tool to discriminate the studied oils, and permitted to separate the samples into four groups.

The richness of the studied Mediterranean oils in polyunsaturated fatty acids (PUFA) and in bioactive compounds such as tocopherols, phytosterols, and polyphenols could be an interesting nutritional tool in preventing and/or attenuating age-related diseases often associated with nutrients deficiency.

Author Contributions

Investigation, Leila Rezig, Lucy Martine, Thomas Nury, Kamel Msaada, Nesrine Mahfoudhi, Philippe Durand, Imen Ghzaeil, and Emmanuelle Prost-Camus; Methodology, Leila Rezig, Lucy Martine, Niyazi Acar, Emmanuelle Prost-Camus, Anne Vejux, and Gérard Lizard; Validation, Leila Rezig, Niyazi Acar, Norbert Latruffe, Adil El Midaoui, Anne Vejux, and Gérard Lizard; Writing, Leila Rezig and Gérard Lizard; Original Draft Preparation, Leila Rezig and Gérard Lizard; Project administration, Leila Rezig and Gérard Lizard; Conceptualization and Supervision, Leila Rezig and Gérard Lizard. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors acknowledge Dr Michel Prost (Laboratoire Spiral Couternon, France) for his expertise for the KRL assay and polyphenols analysis, the association "Mediterranean Nutrition and Health; NMS: Nutrition Méditerranéenne & Santé", and ABASIM (Association Bourguignonne pour les Applications des Sciences de l'Information en Médecine, Dijon, France).

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