

Article

Utilization of Blackthorn Plums (*Prunus spinosa*) and Sweet Cherry (*Prunus avium*) Kernel Oil: Assessment of Chemical Composition, Antioxidant Activity, and Oxidative Stability

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Abstract: *Prunus avium* L. and *Prunus spinosa* L. are valuable fruit-bearing trees known for their bioactive compounds and medicinal properties. However, limited research exists regarding their kernel oils. This study aimed to compare the chemical composition, quality parameters, and bioactive potential of the kernel oils extracted from *Prunus avium* L. and *Prunus spinosa* L. The kernel oils' fatty acid and tocopherol profiles were characterized, and the presence of bioactive compounds were identified and quantified. Total polyphenol content (TPC) and antioxidant activity (AAC) were also measured, indicating the presence of bioactive compounds in both oils. Additionally, the main quality parameters, including oxidative status, were evaluated. The fatty acid analysis revealed a higher proportion of polyunsaturated fatty acids compared to monounsaturated fatty acids in both kernel oil samples. Linoleic acid (57–64%) and oleic acid (18–29%) were the major fatty acids in both *Prunus avium* L. and *Prunus spinosa* L. kernel oils. α -Eleostearic acid (11.87%) was quantified only in *Prunus avium* kernel oil. Furthermore, the α -, β -, γ -, and δ -tocopherol content were determined, and it was found that both kernel oils contained γ -tocopherol as the major tocopherol (~204–237 mg/Kg). TPC in *Prunus avium* L. kernel oil was measured at 9.5 mg gallic acid equivalents (GAE)/Kg and recorded as ~316% higher TPC than *Prunus spinosa* L. kernel oil. However, the recorded AAC were 11.87 and 14.22 μ mol Trolox equivalent (TE)/Kg oil, respectively. Both oils recorded low peroxide values (~1.50 mmol H₂O₂/Kg), and low TBARS value (~0.4 mmol malondialdehyde equivalents, MDAE/Kg oil), but high *p*-anisidine value (23–32). The results indicated that both *Prunus avium* L. and *Prunus spinosa* L. kernel oils exhibited unique chemical compositions.

Keywords: *Prunus spinosa*; *Prunus avium*; kernel oil; fatty acids; polyphenol content; carotenoids; oxidative stability; GC-FID; HPLC-DAD



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1. Introduction

The genus *Prunus*, family Rosaceae, comprises 200 species, including almonds, plums, apricots, peaches, cherries, etc. [1]. Most members of this genus are economically important fruits [2]. Their cultivation occurs all over the world, with Europe being one of the top producers [3]. *Prunus* species are all immensely common temperate fruits that are highly valued and researched primarily for their taste, color, and sweetness, along with their nutritional and bioactive characteristics that are advantageous to human health [4,5].

Prunus avium L. (i.e., sweet cherry) is a member of the family Rosaceae and subfamily Prunoideae [6]. It is a fruit that has been cultivated since ancient times and is widespread in the world [7] with the main countries of cherry production being Turkey, the United States, Iran, Italy, and Spain [8]. In addition to its high-water content, it has a high content of saccharides (mostly glucose, followed by fructose, sorbitol, and sucrose) [9]. Furthermore, cherries are a good source of vitamins A, B, and C and organic acids, with

malic acid being the predominant one [10]. Apart from vitamins, it contains considerable amounts of minerals, with potassium, magnesium, phosphorus, and calcium being the most prevalent [6]. It is noteworthy that *P. avium* fruits are one of the richest sources of phenolic compounds known for physical anticancer and anti-invasive activities [11]. Finally, *P. avium* is considered among the most utilized fruits since it is cultivated for its delicious fruit [8] and there are also varieties cultivated as ornamental trees.

Regarding *Prunus spinosa* L. (i.e., blackthorn plums), it is considered an exotic fruit, and it also belongs to the family Rosaceae and subfamily Amygdaloideae [12,13]. It is primarily found in Europe, western Asia, and regionally in northwestern Africa, but has also been recorded in New Zealand, Tasmania, and the Pacific Northwest and New England regions of the United States [14]. The flesh contains vitamins, such as vitamins C and E, and is also high in minerals, with potassium, calcium, and magnesium being dominant in order of increasing content [15,16]. In addition, it is widely reputed for its antioxidant, antidiabetic, antimicrobial, anti-inflammatory, and anticancer activity and features an abundance of polyphenols such as anthocyanins, phenolic acids, and flavonoids [17–20]. *P. spinosa* itself is also quite a valuable fruit as in addition to its flesh used for fresh consumption, its leaves are often used as a tea substitute [21]. Moreover, concerning its flowers, they exude a liquid with therapeutic properties that are indicated in treating urinary tract disorders [22]. Meanwhile, they are considered harmless for consumption since after cytotoxicity tests, the viability of human peripheral blood mononuclear cells seems unaffected [22].

Oil extracted from plant kernels has gained significant attention due to its various applications in the food, pharmaceutical, and cosmetic industries [23]. More precisely, kernel oils are distinguished for their health properties since they enhance the immune system, by reducing cholesterol levels in the blood and increasing the elasticity of the blood vessel wall [24]. Additionally, kernel oils are known to have antioxidant and antimicrobial properties that are extremely useful, especially in the pharmaceutical industry [25]. Meanwhile, the oxidative stability of kernel oils is excellent due to their high content of carotenoids (provitamin A), also known as retinol [24]. Retinol is frequently used in cosmetics and is highly desirable due to its anti-aging properties [26]. Nevertheless, further research and tests are required in order to identify and quantify all of the properties of kernel oils and properly exploit them. In this way, a circular economy system is being created and profitable products are made from fruit parts that are considered waste.

Although numerous studies have explored the bioactive compounds and medicinal properties of *Prunus avium* L. and *Prunus spinosa* L., the number of studies focusing on the oil obtained from the kernels are scanty and sparse. There is a lack of comprehensive investigations into the composition and quality parameters of these oils. Thus, a research gap exists, necessitating a detailed analysis to fill this gap and explore the untapped potential of these oils. The primary aim of this study is to investigate and compare the oil extracted from the kernels of *Prunus avium* L. and *Prunus spinosa* L. with a focus on their chemical composition, quality parameters, and potential bioactivities. The study aims to characterize the fatty acid and tocopherol profiles, identify and quantify bioactive compounds, and evaluate their main quality parameters and oxidative status activities. Understanding the chemical constituents and unique properties of the kernel oils of *Prunus avium* L. and *Prunus spinosa* L. can open new avenues for their utilization and contribute to the development of innovative products.

2. Materials and Methods

2.1. Reagents

Malondialdehyde and tocopherol standards were from Merck Ltd. (Darmstadt, Germany). Ammonium iron (II) sulfate, trichloroacetic acid, hydrochloric acid (37%), thiobarbituric acid, gallic acid monohydrate, Folin–Ciocalteu reagent, and glacial acetic acid were obtained from Panreac (Barcelona, Spain). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was from Glentham Life Sciences (Corsham, UK). Isooctane, ethyl acetate, and dichloromethane were purchased from Carlo Erba (Vaul de Reuil, France). Cy-

clohexane, *n*-hexane, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2-propanol, methanol, and *p*-anisidine were bought from Sigma-Aldrich (Burlington, MA, USA). Hydrogen peroxide (35%) was obtained from Chemco (Malsch, Germany). Chloroform, sodium carbonate anhydrous, and ammonium thiocyanate were all from Penta (Prague, Czech Republic). Ethanol (99.8%) was bought from Fischer Scientific (Loughborough, UK).

2.2. Samples Preparation

Blackthorn plums (*Prunus spinosa* L.) were harvested from wild plants in a steep location near Spathades, Kalambaka, central Greece (39.7074, 21.7129), and sweet cherries (*Prunus avium* L.) were harvested from Agia, Larissa, Central Greece (39.7246, 22.7346), respectively. The respective coordinates (latitude, longitude) of the two areas were obtained with the assistance of Google EarthTM, version 7.3.2.5776. Both fruits were harvested manually at their peak ripeness and then frozen until examination.

The kernels from each fruit were removed and were washed from any flesh residues. Thirty grams of each *Prunus*' grounded kernels were stirred with *n*-hexane for 6 h. All extractions were conducted using a constant liquid-to-solid ratio of 20 mL/g, and a stirring speed of 500 rpm at room temperature. After extraction, the mixture was centrifuged for 5 min at 4500 rpm. The supernatant was retracted, and the solid residue was re-extracted a second time. Finally, the supernatants were pooled, and the solvent was removed by vacuum evaporation. The extracted oil samples were stored in dark bottles at 4 °C for further analysis.

2.3. Oil Fatty Acid Composition

The procedure for preparing fatty acid methyl esters (FAMES) from oils was in accordance with Annex XB of Commission Regulation (EC) No. 796/2002 [27]. An Agilent Technologies (Santa Clara, CA, USA) gas chromatograph model 7890A was employed, with an installed Omegawax capillary column (30 m × 320 µm × 0.25 m) (Supelco, Bellefonte, PA, USA). The flow rate was 1.4 mL/min with helium as the carrier gas. The column temperature program was as follows: isotherm for 5 min at 70 °C, then ramped to 160 °C at a rate of 20 °C/minute, then rose to 200 °C at a rate of 4 °C/minute, and finally topped at 240 °C at a rate of 5 °C/minute. The injector and flame ionization detector (FID) temperatures were kept at 240 and 250 °C, respectively. The hydrogen flow rate was 50 mL/min, the air flow rate was 450 mL/min, and the helium makeup flow rate was 50 mL/min. In split mode (1:100), 1.0 µL samples were injected. Individual peaks were recognized by comparing them to the FAME Mix C8–C24 reference standards (Sigma-Aldrich, St. Louis, MO, USA). The percentage composition of the samples was calculated using the normalization technique (without correction factors) from the GC peak regions. Percentages of components were averaged from triplicate GC-FID analyses.

2.4. Oil Tocopherol Determination

The procedure employed for the quantification of tocopherol was a modified version of the approach initially described by Lalas et al. [28]. The analysis was conducted using a Shimadzu CBM-20A high-performance liquid chromatograph (HPLC) (Shimadzu Europa GmbH in Duisburg, Germany). The HPLC system was equipped with a SIL-20AC autosampler and a CTO-20AC column oven. The detection process was conducted utilizing a Shimadzu RF-10AXL fluorescence detector, with excitation set at 294 nm and emission at 329 nm. The column employed in this study was a Waters µ-Porasil column with a pore size of 125 Å, particle size of 10 µm, and dimensions of 3.9 mm × 300 mm (Waters Corporation, Milford, MA, USA). The mobile phase utilized in the experiment was composed of a mixture of *n*-hexane, 2-propanol, and absolute ethanol in a ratio of 97.5:2.0:0.5 *v/v*, respectively. The mobile phase was delivered at a flow rate of 1 mL/min. The procedure for sample preparation was as follows: An amount of 0.5 g of oil was weighed and placed into a volumetric flask with 5 mL of hexane and was appropriately diluted. A sample of 20 µL was injected into the HPLC

system. The determination of tocopherol content (TC) was carried out by calculating the mg of each tocopherol per Kg of oil, using the following equation:

$$\text{TC (mg T/Kg Oil)} = \frac{C_T \times V \times 1000}{w} \quad (1)$$

2.5. Determination of Total Polyphenol Content (TPC)

Polyphenol extraction from oils was carried out in accordance with a previously described procedure [29], and the Folin–Ciocalteu determination was carried out using a known methodology [30]. A quantity of 1 g of oil was diluted with 2 mL of n-hexane and extracted with 2 mL of a 60% methanol/water combination. The mixture was forcefully shaken using a Vortex apparatus before being centrifuged at 4500 rpm for 5 min. The reaction was then allowed to proceed for 2 min after 0.1 mL of the aqueous phase and 0.1 mL of the Folin–Ciocalteu reagent were transferred to a 1.5 mL Eppendorf tube. After adding 0.8 mL of 5% *w/v* sodium carbonate solution, the mixture was heated in a thermostatic water bath (Falc Instruments LBS2, Treviglio, Italy) for 20 min at 40 °C. A Shimadzu UV-1700 PharmaSpec spectrophotometer (Kyoto, Japan) was used to measure absorbance at 740 nm, and a gallic acid calibration curve (10–100 mg/L) was used to calculate total polyphenol concentration. Total polyphenols (TPC) in the oil were reported as mg gallic acid equivalents (GAE) per kilogram of oil.

2.6. Evaluation of DPPH• Antiradical Activity (AAC)

Antiradical activity from oil samples was calculated as previously published [29]. In brief, 5 mL of ethyl acetate was mixed with 0.5 g of oil in a 5 mL volumetric flask. Then, 50 µL of the oil sample was combined with 950 µL of DPPH• solution (100 µM in ethyl acetate) and vigorously shaken for 10 s. The absorbance at 515 nm was measured immediately after mixing ($A_{515(i)}$) and 30 min later ($A_{515(f)}$). The antiradical activity was calculated using the following equation:

$$\text{Inhibition (\%)} = \left(\frac{A_{515(i)} - A_{515(f)}}{A_{515(i)}} \right) \times 100 \quad (2)$$

The results were reported as the Trolox equivalent antioxidant capacity (TEAC) using a calibration curve (50–500 µM) produced by plotting % inhibition versus Trolox concentration (in µM).

2.7. Determination of Total Carotenoids Content (TCC)

Carotenoid determination was performed as reported previously [31], but with modifications. A single drop of oil sample (~0.01 g) was filled with cyclohexane in a 5 mL volumetric flask. The absorbance was recorded at 450 nm using a 1 cm quartz cell spectrophotometrically. The total carotenoid concentration (C_{TCn}) was evaluated with the following equation:

$$C_{TCn} (\mu\text{g/mL Oil}) = \frac{A \times \text{FD}}{A^{1\%}} \times C^{1\%} \quad (3)$$

where A is the measured absorbance at 450 nm, FD is the dilution factor, $A^{1\%} = 2500$ and $C^{1\%} = 10,000$ µg/mL. Extraction yield in TCn (Y_{TCn}), represented as β-carotene equivalents (CtE), was then determined:

$$Y_{TCn} (\text{mg CtE/Kg Oil}) = \frac{C_{TCn} \times V}{w} \quad (4)$$

where V is the volume of the cyclohexane phase (in mL) and w is the amount of oil (in Kg).

2.8. Oil Stability Tests

2.8.1. Peroxide Value (PV) Assay

The IDF standard technique 74A:1991 [32] was used with certain modifications to determine the peroxide levels of kernel oil samples. In a 2 mL Eppendorf tube, 0.05 g of oil sample was dissolved in 2 mL dichloromethane/ethanol (3:2 *v/v*) on a vortex mixer for 2–4 s. A quantity of 20 μL of oil extract was combined with 1960 μL of solvent (dichloromethane/ethanol). A quantity of 10 μL of ammonium thiocyanate solution (4 M in water) was added, and the sample was stirred for 2–4 s on a vortex mixer. The sample was then combined for 2–4 s on a vortex mixer with 10 μL of ammonium iron(II) sulfate solution (25.5 mM in 10 M HCl). After 5 min at room temperature, the absorbance of the sample was measured at 500 nm against a blank solution (i.e., reaction mixture without lipid) using a UV spectrophotometer. The PV was calculated using a hydrogen peroxide (H_2O_2) calibration curve created by repeating the technique above at six different concentrations (50–500 $\mu\text{mol/L}$ in dichloromethane/ethanol). Using the following equation, the PV was calculated as mmol H_2O_2 per Kg of oil:

$$\text{PV (mmol H}_2\text{O}_2/\text{Kg Oil)} = \frac{C_{\text{H}_2\text{O}_2} \times V}{w} \quad (5)$$

where $C_{\text{H}_2\text{O}_2}$ is the H_2O_2 concentration (in $\mu\text{mol/L}$), V is the extraction medium volume (in L), and w is the weight of the reaction oil sample (in g).

2.8.2. Thiobarbituric Acid Reactive Substances (TBARS) Assay

The TBARS assay was performed in accordance with Qiu et al. [33]. In a tube, 0.1 g of oil sample was mixed with 5 mL of thiobarbituric acid (TBA) solution (consisting of 15 g of trichloroacetic acid, 0.375 g of TBA, and 1.76 mL of 12 M HCl in a 100 mL volumetric flask and filling the rest with deionized water). The mixture was forcefully shaken and incubated at 95 $^\circ\text{C}$ for 20 min. Following incubation, samples were put in a cold bath for 5 min. The mixture was vortexed and centrifuged at 4500 rpm for 10 min after 200 μL of chloroform was added. A UV spectrophotometer was used to measure the absorbance of the supernatant at 532 nm. By replacing the sample with deionized water, a blank solution was created. The TBA was calculated as mmol of malondialdehyde equivalents (mmol MDAE) per kg of oil using a malondialdehyde calibration curve (15–300 $\mu\text{mol/L}$ in deionized water) using the equation below:

$$\text{TBA}_{\text{value}} \text{ (mmol MDAE/Kg Oil)} = \frac{C_{\text{MDA}} \times V}{w} \quad (6)$$

where C_{MDA} denotes the malondialdehyde concentration (in $\mu\text{mol/L}$), V is the volume of the extraction medium (in L), and w is the weight of the oil sample (in g).

2.8.3. *p*-Anisidine Value (*p*-AV) Assay

The ES ISO 6885:2012 method [32] was used to calculate the *p*-anisidine value (*p*-AV). Isooctane was added to 0.5 g of oil sample in a 10 mL volumetric flask. A volume of 1 mL of the diluted oil sample solution was transferred to an Eppendorf tube, and 0.2 mL of glacial acetic acid was added and forcefully shaken. After 10 min in the dark, absorbance (A_0) at 350 nm was measured. Furthermore, 1 mL of the diluted oil sample solution was mixed with 0.2 mL of *p*-anisidine analytical reagent (0.5% in glacial acetic acid) and rapidly shaken. After 10 min in the dark, the solution's absorbance (A_1) was measured at 350 nm. Then, 1 mL of isooctane was added to 0.2 mL of *p*-anisidine analytical reagent, shaken swiftly and after 10 min in the dark, absorbance (A_2) at 350 nm was measured. The following equation was used to determine the *p*-AV:

$$p\text{-AV} = \frac{100 Q V}{m} 0.24[(A_1 - A_2 - A_0)] = 12 \left(\frac{A_1 - A_2 - A_0}{m} \right) \quad (7)$$

where Q is the sample content of the measured solution, in grams per milliliter ($Q = 0.05$ g/mL); V is the volume in which the test sample is dissolved, in milliliters ($V = 10$ mL); m is the mass of the test portion, in grams; A_0 is the absorbance of the unreacted test solution; A_1 is the absorbance of the reacted solution; A_2 is the absorbance of the blank; and 0.24 is the correction factor for the dilution of the test solution with 0.2 mL of reagent or glacial acetic acid (+20%).

2.8.3. Conjugated Dienes (CD) and Trienes (CT) Determination

Pegg et al. [34] described a technique for measuring the values of conjugated dienes and trienes. Briefly, 0.01 g of oil sample was added to a 5 mL volumetric flask, along with cyclohexane. The absorbance of conjugated dienes and trienes was measured at 232 nm and 270 nm, respectively. The conjugated diene (CD) and triene (CT) values were computed using the formulae below:

$$C_{CD} \text{ (mmol/mL)} = \frac{A_{232}}{\epsilon \times l} \quad CD_{\text{value}} \text{ (mmol/Kg Oil)} = \frac{C_{CD} \times (5 \times 10^3)}{w} \quad (8)$$

$$C_{CT} \text{ (mmol/mL)} = \frac{A_{270}}{\epsilon \times l} \quad CT_{\text{value}} \text{ (mmol/Kg Oil)} = \frac{C_{CT} \times (5 \times 10^3)}{w} \quad (9)$$

where C_{CD} and C_{CT} are the CD and CT concentrations, respectively, in M (molar concentration); A_{232} and A_{270} are the absorbances of the lipid solution at 232 nm and 270 nm, respectively; ϵ is the molar absorptivity of linoleic acid hydroperoxide (2.525×10^4 M⁻¹ cm⁻¹); l is the path length of the cuvette in cm (1 cm); 5×10^3 is a factor that encompasses the volume of solvent (5 mL) used to dissolve the oil sample; and w is the weight of the oil sample in g, so that the contents of CDs and CTs can be expressed in mmol per Kg of oil.

2.8.4. Totox Value (TV) Assay

The Totox value was estimated using the method described by Galanakis et al. [35]. The Totox value (TV) is a measure of the total oxidation that takes into account both primary and secondary oxidation products. It is the sum of the PV and the p -AV:

$$TV = 2 \times PV + p\text{-AV} \quad (10)$$

2.9. Statistical Analysis

The analyses were carried out in triplicate, standard deviation was calculated, and the data were reported as mean values of the triplicate analyses \pm standard deviation. A one-way analysis of variance (ANOVA) test was used to determine the statistical significance of differences in mean values; $p < 0.05$ was considered statistically significant. JMP[®] Pro 16 software (SAS, Cary, NC, USA) was used for associated statistics.

3. Results & Discussion

A comprehensive analysis and comparison between oils derived from the kernels of *Prunus spinosa* L. (PSKO) and *Prunus avium* L. (PAKO) was carried out, so as to investigate whether the extracted oils are suitable for industrial uses.

3.1. Oil Fatty Acid Composition

Fatty acids are molecules that exhibit biological activity and possess an extensive variety of beneficial health effects. For instance, polyunsaturated and monounsaturated fatty acids were found to prevent cardiovascular diseases [36]. Plant-based fats, such as nuts and vegetable oils, are associated with improved gut microbial diversification [37]. PSKO and PAKO were examined for their composition in fatty acids and the results are presented in Table 1. In the present study, the percentages of fat in PAKO and PSKO samples that were detected were 8.5 and 7.43%, respectively. Fatty acid concentration is regarded among the most crucial variables influencing the nutritional value and stability of edible oil [38].

It is noteworthy that the consumption of monounsaturated and polyunsaturated fatty acids reduces low-density lipoprotein and shows a positive effect on the brain system [39]. In addition, in both samples C18:1 (oleic acid) and C18:2 (linoleic acid) were the most prevalent fatty acids in both kernel oils. Their presence is important since C18:1 fatty acid is known to exhibit anticancer and anti-atherogenic effects [40], whereas C18:2 fatty acid is beneficial for preventing both metabolic and cardiovascular diseases [41]. Some additional important fatty acids were found in PAKO, such as the two C18:3 (omega-3) isomers (linolenic acid and α -eleostearic acid), with the latter measured in remarkable amounts of ~11% compared to the linolenic acid (~1%). This finding does not come as a surprise as certain plant kernel oils are known to be the richest natural sources of these isomers [42]. C18:3 (omega-3) isomers are also known for their beneficial effects on human health and their anticancer properties [43]. Comparing the value of the C18:3 (ω -3) isomer and C22:0 (behenic acid) fatty acids with a previous study [44], the results are comparable. In particular, the amounts detected were 11.52 and 0.15% for the C18:3 (ω -3) isomer and C22:0, respectively [44]. Meanwhile, in the same study, after examining these two fatty acids in PSKO kernel, quite small amounts of 0.03 and 0.07% were detected in the C18:3 (ω -3) isomer and C22:0, respectively.

Table 1. Fatty acid composition of PSKO and PAKO.

Fatty Acids (%)	PSKO	PAKO
C16:0	6.04 \pm 0.15 ^b	7.34 \pm 0.5 ^a
C18:1	29.35 \pm 1.29 ^a	18.57 \pm 0.97 ^b
C18:2 (ω -6)	64.4 \pm 3.28 ^a	57.86 \pm 2.37 ^b
C20:0	nd *	2.78 \pm 0.06
C18:3 (ω -3)	0.21 \pm 0.01 ^b	1.42 \pm 0.07 ^a
C18:3 (ω -3) isomer	nd	11.87 \pm 0.58
C22:0	nd	0.16 \pm 0
Σ SFA ¹	6.04 \pm 0.15 ^b	10.28 \pm 0.56 ^a
Σ MUFA ²	29.35 \pm 1.29 ^a	18.57 \pm 0.97 ^b
Σ PUFA ³	64.61 \pm 3.29 ^a	71.15 \pm 3.02 ^a
Σ UFA ⁴	93.96 \pm 4.58 ^a	89.72 \pm 3.99 ^a
PUFA: SFA ratio	10.7 \pm 0.28 ^a	6.92 \pm 0.09 ^b
MUFA: PUFA ratio	0.45 \pm 0 ^a	0.26 \pm 0 ^b
ω -6:3 ratio	303.69 \pm 3.96 ^a	4.36 \pm 0.04 ^b
COX ⁵	6.97 \pm 0.35 ^a	9.02 \pm 0.39 ^b

Different superscript letters (e.g., a, b) indicate statistically significant differences ($p < 0.05$) within each line. * nd: not detected. ¹ SFAs, saturated fatty acids (%): SUM of C16:0, palmitic acid; C20:0, arachidic acid; C22:0, behenic acid. ² MUFAs, monounsaturated fatty acids (%): SUM of C18:1, oleic acid. ³ PUFAs, polyunsaturated fatty acids (%): SUM of C18:2, ω -6, linoleic acid; C18:3, ω -3, linolenic acid; C18:3 (ω -3) isomer, α -Eleostearic acid. ⁴ UFAs, SUM of MUFAs and PUFAs. ⁵ COX, calculated oxidizability value.

A key goal of this research is to examine the potential of these oils to be used either for consumption or in other industrial sectors such as cosmetics or pharmaceuticals. The most widely consumed oils include sunflower, almond, and olive oil. Analyzing these oils for their fatty acid composition yields remarkable results. For example, C22:0 is found only in olive oil in an amount of 0.15%, similar to the amount found in PAKO (0.16%). While regarding the C18:3 (omega-3) isomer, it has not been detected in any of the edible oils, apart from PAKO studied in the present study. Moreover, omega-6 fatty acid in sunflower oil was recorded at 59.7%, a close and comparable value to PAKO and PSKO. Finally, C18:1 was detected in amounts of 12.7, 67.2, and 66.4% in sunflower, almond, and olive oils, respectively. While at the same time, the values in PAKO and PSKO, were 18.57 and 29.35%, respectively [45]. Therefore, it is understood that the two oils concerned, PAKO and PSKO, are completely comparable to the edible oils mentioned above, in particular PAKO. Obviously, PAKO can even be compared to olive oil in terms of certain major fatty acids.

In addition, the unsaturated fatty acid index was also evaluated, which refers to unsaturated fatty acids and is associated with positive health effects [46]. Both kernel samples have high unsaturated fatty acid content with *P. spinosa* exhibiting a slightly higher value ($p > 0.05$) compared to *P. avium*. Furthermore, the ω -6:3 ratio is also an important parameter to take into account, with the ratio of 1:1 to 5:1 being considered the most beneficial for human health [47]. According to our results, PSKO had a significantly higher value that far exceeds these ratios (~ 300), whereas the ratio measured in PAKO (~ 4) is within the above limit. This outcome makes PSKO less beneficial for consumption, albeit, not unsuitable. Finally, the COX values were calculated. The lower the COX value, the better the oxidative stability of the oil, and thus, a longer shelf life [48]. According to the results, it is clear that the COX values are quite high, with PAKO being even higher. Taking also into account the ω -6:3 ratio results, it can be inferred that the consumption of this oil may not be recommended. However, this does not rule out the possibility of using these kernels' oils in other industries for the production of non-consumable products, such as the cosmetics industry.

3.2. Oil Tocopherol Determination

Tocopherols are lipophilic molecules that possess biological activity, comprising four homologous variants, namely α -, β -, γ -, and δ -tocopherol. Tocopherols possess antioxidant properties and exhibit remarkable efficacy in the context of oxidative reaction chain-breaking, making them highly suitable for deployment as food additives [49]. The order of relative antioxidant activity among the tocopherol homologs is $\alpha > \beta > \gamma > \delta$ [50]. The results of the determined tocopherols in both oils are presented in Table 2. The overall concentration of tocopherols exhibited a range of 240–260 mg/Kg of each oil. The most prevalent tocopherol in both oils was γ -tocopherol. In fact, PAKO had ~ 237 mg/Kg of this tocopherol and was statistically significant ($p < 0.05$), and was higher than PSKO by $\sim 16\%$. An interesting finding was that PSKO had 30.6 mg/Kg of α -tocopherol and it was almost twice the amount found in PAKO. In PAKO and PSKO, low δ -tocopherol values were recorded which were measured as ~ 6 mg/Kg. Our results are comparable with Aqil et al. [51] who studied the kernel oil from four *Prunus avium* L. varieties from Morocco. They found that γ -tocopherol was the most prevalent in all four varieties. In addition, the *Cœur de Pigeon* variety recorded ~ 33 mg/Kg of α -tocopherol, ~ 300 mg/Kg of γ -tocopherol, and ~ 18 mg/Kg of δ -tocopherol, which were similar to our findings. In the same study [51], corn and olive oil had a $(\gamma + \delta)/\alpha$ tocopherol ratio of 1.32 and 0.43, respectively. Ratios close to or below 1 could indicate higher antioxidant activity. In other words, it means that the higher the α -tocopherol content, the higher the antioxidant activity of the sample. In our study, the $(\gamma + \delta)/\alpha$ tocopherol ratio of the two oils showed statistically significant differences ($p < 0.05$). PSKO had 6.8 and PAKO had $\sim 122\%$ increased ratio.

Table 2. Tocopherol content of PSKO and PAKO.

Tocopherols (mg/Kg)	PSKO	PAKO
α -tocopherol	30.6 ± 2.1^a	16.2 ± 0.7^b
β -tocopherol	not detected	not detected
γ -tocopherol	204.5 ± 10.2^b	237.3 ± 11.9^a
δ -tocopherol	4.8 ± 0.3^b	6 ± 0.1^a
Total	239.9 ± 12.6^a	259.5 ± 12.7^a
$\frac{\gamma + \delta}{\alpha}$ tocopherol ratio	6.8 ± 0.1^b	15.1 ± 0.1^a

Different superscript letters (e.g., a, b) indicate statistically significant differences ($p < 0.05$) within each line.

3.3. Polyphenol Content, Carotenoids Content and Antioxidant Activity

Polyphenols are a category of bioactive compounds that are found in abundance in various plant-based foods and their derived products. The biological functions of polyphenols have been extensively studied through various in vitro and in vivo experimental models [52,53]. These compounds have several health-promoting qualities and may prevent,

postpone, or alleviate chronic illnesses such as cardiovascular, inflammatory, and neurological disorders [54]. Overall, kernel fats and oils are considered poor in polyphenols. According to the results in Table 3, PSKO contained 3.33 mg/Kg polyphenols while the PAKO contained 9.44 mg/Kg polyphenols, i.e., the latter had a 183.48% higher amount. PAKO had the highest polyphenol content of the samples tested, with a statistically significant difference ($p < 0.05$). The significant difference in polyphenols in PAKO and PSKO may be due to genetic variations. The two plant species belong to the same genus (*Prunus*) but are different species. Genetic variation can lead to differences in the production and accumulation of phenolic compounds in their kernels. Various factors, such as cultivar type, growing conditions, and environmental factors, can influence the expression of genes responsible for the synthesis of phenolic compounds, resulting in varying TPC levels. This outcome was quite expected as previous research has reported that cherries are rich in polyphenols in all parts of it including the flesh, and by-products such as kernel [55], while *P. spinosa* is rich in polyphenols only in its flesh [56]. As previously mentioned, apricot is a fruit that also belongs to the genus *Prunus* [57]. In a previous study, the amount of polyphenols in *Prunus armeniaca* (i.e., apricot) oil was analyzed over two consecutive years, and it was found to contain polyphenols ranging between 7.94 mg/Kg and 8.18 mg/Kg [58]. A quantity that coincides more closely with the quantity detected in PAKO. One more related species is *Prunus serotina* (i.e., almond) [59]. *Prunus serotina* kernel extract turns out to be quite rich in polyphenols since after extraction with water, polyphenols were found to be 20 mg/Kg while after extraction with acetone, they were found to be 470 mg/Kg [60]; these quantities are much higher than those recorded in the two species studied in our research. This means that depending on the species of the genus *Prunus*, the total phenolic content varies. Comparing the results of the present study with values recorded in virgin olive oil and one more widely used oil, coconut oil, the following results were observed. In virgin oil, the TPC values were 500 mg/Kg [61] while in coconut oil 70–300 mg/Kg [62] were recorded. Consequently, PAKO and PSKO are classified as extremely low in total polyphenols. However, given the importance of polyphenols for human health, future studies are needed to identify specific polyphenols in both oils.

Table 3. Total polyphenol content (TPC), total carotenoid content (TCC), and antiradical activity (AAC) of PSKO and PAKO samples.

Index	PSKO	PAKO
TPC (mg GAE/Kg oil)	2.28 ± 0.82 ^b	9.5 ± 1.47 ^a
TCC (mg CtE/Kg oil)	218.62 ± 2.75 ^a	40.78 ± 1.94 ^b
AAC (μmol TE/Kg oil)	14.22 ± 0.74 ^a	11.87 ± 0.35 ^b

Different superscript letters (e.g., a, b) indicate statistically significant differences ($p < 0.05$) within each line.

Carotenoids are compounds responsible for the pigmentation of numerous plants, fruits, and flowers. Among the main carotenoids known to promote human health is β -carotene [63]. Examples of the benefits of β -carotene include the prevention of certain cancers, protection of the gastric mucosa from ulcers, the ability to prevent photosensitization in skin conditions, and increasing the immune response to infections [63]. In addition, beta-ionone containing carotenoids (i.e., mostly carotenes and cryptoxanthin) have the ability to be converted into retinol (provitamin A activity), and it also possesses anti-aging properties [63,64]. In the present study, the total carotenoid content in the kernel oil of *Prunus* fruits was studied and the results are presented in Table 3, where PSKO was found to contain 218.62 mg/Kg while in PAKO, the content was 436.09% less. Thus, it becomes clear that the oil from PSKO is extremely rich in carotenoids and all its beneficial properties, making it highly useful in the food and cosmetic industry. Following previous research [65] examining the total carotenoids in the corresponding PAKO, quite low values were recorded, classifying this oil as poor in carotenoids. However, future studies dedicated to examining the carotenoid content in these kernel oils would provide valuable insights into their nutritional profile and potential contributions to meeting vi-

tamin A requirements. Conclusively, studying a different species from the genus *Prunus*, apricot—*P. armeniaca*, values were recorded in the kernel oil between 42.3 and 66.8 mg/Kg, which are values similar to those of PAKO. Taking into account both polyphenols and total carotenoids, it is obvious that the oil from the *P. avium* and *P. armeniaca* have similar nutritional properties [66]. Following research conducted on soy oil and olive oil, the value of total carotenoids was recorded around 0.12 mg CtE/Kg in both of them [67]. These quantities are statistically significantly lower values compared with the tested PAKO and PSKO, consequently listing the two examined oils as having excellent total carotenoid content. Lastly, by comparing the total carotenoid content in the kernel of a similar species to *P. avium* and *Prunus cerasus*, the amount of 1.7–3.9 mg/Kg was determined, which is statistically much lower than that found in the two oils tested. The difference may be due to the fact that the previous study examined the kernel as such and not the oil, which may have had an even lower amount [68]. The presence of bioactive compounds such as tocopherols and carotenoids in the kernel oils may have positive implications for skin health. These compounds possess antioxidant and anti-inflammatory properties, making them valuable ingredients for skincare products.

In view of the essential role of antioxidants for food, pharmaceuticals, and cosmetics [65], the antioxidant activity of the oils was also assessed in the present study. In more detail, antioxidants protect against inflammation by scavenging free radicals, which play an important role in diseases such as cancer and Alzheimer's [69]. Additionally, antioxidants work by neutralizing free radicals which reduces the aging process, and aids against the damage caused by radicals in the skin [70]. Their determination was performed using the DPPH method and the activity was expressed in μg Trolox equivalents (TE)/Kg oil. According to Table 3, in PSKO, 14.56 μmol TE/Kg was recorded, which means PSKO is richer in antioxidant activity than PAKO up to ~20%. The difference between the two samples is considerable, since the new products that can be developed using this KO will enjoy the benefits of this antioxidant activity. This outcome was expected since the PSKO was notably higher in total carotenoids, which are known for their antioxidant activity [71]. Moreover, in Table 2 it was previously mentioned that PSKO had twice the amount of α -tocopherol than PAKO, supporting this finding. In a previous study evaluating the antioxidant activity in the bark of *Prunus padus* L., about 35.56 μmol TE/Kg was found [72]. This amount is 144% higher than the highest antioxidant activity value recorded in our results. However, it was expected that a higher amount of antioxidants was recorded in the bark compared to the kernel oil of fruits belonging to the same genus. The present study is the first attempt to isolate bioactive compounds and quantify antiradical activity of *Prunus* kernel oils by this approach, initiating further research on *Prunus* fruit oils, their antioxidant properties, and their potential utilization. The above-mentioned bioactive compounds in *Prunus avium* L. and *Prunus spinosa* L. kernel oils, hold diverse potential health implications and offer various practical applications. Their antioxidant, anti-inflammatory, cardioprotective, and anticancer properties make them valuable candidates for promoting human health and well-being. Furthermore, the incorporation of oils into food and cosmetic products can provide natural sources of essential nutrients and antioxidants, enhancing the appeal of these products to consumers.

3.4. Oxidative Status Indices

The primary oxidation process begins with the absorption of oxygen within the oil mass. During this process, depletion of reactants, such as unsaturated fatty acids or oxygen, and generation of primary oxidation products, such as hydroperoxides and conjugated dienes takes place. These values are frequently employed for assessing the extent of primary oxidation [73]. The spectrophotometric absorption of conjugated dienes is directly proportional to the quantity of hydroperoxides present and exhibits a strong linear correlation with the peroxide value [74]. However, relying solely on techniques that assess the extent of primary oxidation to monitor lipid oxidation is insufficient. The assessment of secondary oxidation levels frequently involves the utilization of small molecular products.

The breakdown of lipid hydroperoxides is known to result in the formation of various carbonyl compounds, hydrocarbons, ketones, and other substances that are responsible for the degradation of food quality. These degradation products can negatively impact the sensory attributes of foods, such as odor, flavor, color, and texture, as well as potentially introduce toxic properties [75]. Malondialdehyde is the primary aldehyde resulting from the oxidation of oil and is commonly evaluated with the TBARS assay [74]. Also, the assessment of other significant aldehydes, such as α,β -unsaturated aldehydes, is typically conducted using the *p*-anisidine value [76]. The *p*-anisidine value is frequently employed in combination with peroxide value measurements to characterize the overall degree of oxidation, as indicated by the Totox value. Nevertheless, the Totox value can be considered a mathematical parameter [77].

The results from the oxidative status parameters examined are shown in Table 4. PAKO was found to have a PV similar to that of PSKO, averaging 1.5 mmol H₂O₂/Kg oil. These findings could be compared with Atik et al. [44,78] who studied physicochemical and bioactive characteristics on both PSKO and PAKO. They measured 0.6 and 0.7 mmol H₂O₂/Kg oil in PSKO and PAKO, respectively. Regarding TBARS, statistically significant differences ($p < 0.05$) were recorded between the two samples, but both values were low (~0.4). Most vegetable oils including sunflower, peanut, palm, camelia, and corn oils have a TBARS value below 0.3 [79]. Hence, if the assessment of lipid oxidation solely relies on the quantification of TBARS and peroxide values, it is possible that a substantial portion of oxidation products may go undetected, leading to a significant underestimation of the degree of lipid oxidation [80]. In this perspective, the results from the *p*-AV highlighted two key points. PAKO had a statistically significant ($p < 0.05$) higher *p*-AV from the other sample. Both oil samples recorded high *p*-AV values, ranging between 23.23 and 32.76. Consequently, PAKO had the highest TV among the two samples, with the value ranging from 26.19 to 35.83. As stated by Gupta [81], the preferred *p*-AV for fresh frying oil should be below 4, while the maximum acceptable value should not exceed 6. Greater values than 6 mean that the oil is significantly oxidized. This high *p*-AV renders the oil samples not preferable for human consumption. A low PV does not necessarily imply that the lipids have not undergone oxidation. The lack of correlation between the PV and the *p*-AV could be attributed to the fact that lipid hydroperoxides do not play a significant role in the development of off-flavors. During fatty acid oxidation, hydroperoxides reach a peak and then start to break down, while secondary oxidation products are continuously produced, correspondingly increasing the *p*-AV [82]. This noteworthy observation was also confirmed by Guillen et al. [83], who reported that in specific oils, such as olive oil and rapeseed oil, the generation of secondary oxidation products begins nearly concurrently with the formation of hydroperoxides. Conversely, in other oils like sunflower and safflower oils, the breakdown of hydroperoxides initiates when the concentration of these compounds reaches a noticeable level. To elucidate this remarkable increase in the *p*-AV, the oxidative stability results that we found could be compared with other edible vegetable oils. Vegetable oils that were exposed to oxidizing conditions reported significantly lower values than we found in our samples. For instance, Siddique et al. [84] evaluated the antioxidant activity of *Moringa oleifera* leaves extract in sunflower oil under accelerated storage conditions. In a control sunflower oil sample, they measured an initial *p*-AV of 4. The *p*-AV reached ~26 and ~35 after 9 and 12 days of consecutive exposure to 65 °C, respectively. Similar work was conducted by Naz et al. [85] who investigated the oxidative stability of vegetable oils, namely olive oil, corn oil, and soybean oil under distinct storage conditions. The *p*-AV from control oil samples initially ranged from 0.06 to 0.11 and after 90 min of frying it reached 3.6–5.6. Besides the oxidation of fatty acids to produce secondary oxidation products, another possible explanation for the elevated *p*-AV would be the presence of another aldehyde in the analyzed kernel oils (i.e., benzaldehyde). This compound contributes an almond-like aroma to the oils and is derived from the enzymatic hydrolysis of amygdalin, which is present (albeit in small amounts) in the kernels of fruits of the genus *Prunus* [86]. However, future studies are needed to examine the presence of amygdalin and other related

cyanogenic glycosides to gain a more comprehensive understanding of the safety aspects of the two oils.

Table 4. Oxidative status indices of *P. spinosa* and *P. avium* kernel oil samples.

Index	PSKO	PAKO
PV (mmol H ₂ O ₂ /Kg oil)	1.48 ± 0.05 ^a	1.53 ± 0.06 ^a
TBARS (mmol MDE/Kg oil)	0.46 ± 0.01 ^a	0.34 ± 0.03 ^b
<i>p</i> -AV	23.23 ± 0.55 ^b	32.76 ± 0.51 ^a
CD _{value} (mmol/Kg oil)	6.81 ± 0.01 ^b	68.94 ± 0.22 ^a
CT _{value} (mmol/Kg oil)	0.64 ± 0.01 ^b	151.11 ± 1.85 ^a
TV	26.19 ± 0.56 ^b	35.83 ± 0.63 ^a

Different superscript letters (e.g., a, b) indicate statistically significant differences ($p < 0.05$) within each line.

Finally, statistically significant differences ($p < 0.05$) were observed in both the CD_{value} and the CT_{value} between the samples. In PAKO, a ~10 times higher CD_{value} than that in PSKO was recorded. Their corresponding difference in the CT_{value} is more than 200 times higher. Both elevated CD and CT values from PAKO could indicate the high oxidation level of the fatty acids. Regarding the difference in the value of conjugated dienes/trienes, a possible explanation could be given in terms of the chemical composition in fatty acids. Polyunsaturated fatty acids are less stable than monounsaturated fatty acids and are more easily conjugated [87]. PSKO has more monounsaturated fatty acids and fewer polyunsaturated ones, while the reverse occurs in PAKO. As mentioned before, PAKO exhibited a higher concentration in PUFAs, which are more susceptible to oxidation and could be responsible for the observed elevation in the CD value. Ben-Ali et al. [88] investigated the stability of sunflower oil under accelerated storage conditions with the use of basil extract. A control sunflower oil sample recorded initially 1 mmol/Kg oil in both the CD_{value} and the CT_{value}, whereas these values were elevated at 25 and 10 mmol/Kg, respectively, when the oil was exposed at 70 °C for 24 days.

4. Conclusions

This study focused on the analysis of chemical characteristics and antioxidant properties of oils derived from the kernel of two *Prunus* species fruits, *Prunus spinosa* L. (blackthorn plums) and *Prunus avium* L. (sweet cherries) cultivated in Greece. The results of the study indicated that both kernels contained oil that contained various compounds of pharmacological interest, which are recognized for their therapeutic properties. Their high oleic and linoleic acid content, in conjunction with their high tocopherol content, entitles these oils to be of exceptional interest in terms of promoting human health. The oils also recorded low PVs and TBARS values, but high *p*-anisidine values, rendering them unsuitable for human consumption. On the other hand, the existence of abundant bioactive compounds, including carotenoids, and polyphenols in the examined oils, indicates the potential for their utilization in the field of cosmetics, thereby providing the possibility of producing enriched beauty products of great commercial and economic interest.

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