

Article

Effect of Extraction Method on the Physicochemical Properties, Bioactive Compounds, and Oxidative Stability of Oils from Rosehip, Japanese Quince, Grape, and Strawberry Seeds

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Featured Application

These results provide practical guidance for selecting extraction procedures aimed at recovering oils from fruit seed by-products for potential use in food, nutraceutical, cosmetic, and analytical applications. Hexane-based methods may be preferable when oxidative quality and stability are priorities, whereas the Folch method can be useful for broader recovery of lipid-associated bioactive compounds, although the resulting oils may require further purification or stabilization.

Abstract

Fruit seeds generated during fruit processing are promising sources of valuable oils and bioactive compounds, but extraction conditions can strongly affect the quality of the recovered lipid fraction. This study compared Soxhlet extraction, room-temperature hexane extraction, and the Folch method for extracting oils from rosehip, Japanese quince, grape, and strawberry seeds. The extracted oils were evaluated for extraction yield, physicochemical quality, fatty acid composition, nutritional lipid indices, pigment content, total phenolic content, antioxidant activity, and oxidative stability measured by pressure differential scanning calorimetry. Oil extraction yield ranged from 4.34% to 10.76% and depended mainly on seed type. All oils were rich in unsaturated fatty acids, with polyunsaturated fatty acids (PUFAs) predominating in rosehip, strawberry, and grape seed oils, whereas Japanese quince seed oils showed the highest monounsaturated fatty acid (MUFA) fraction. The Folch method generally enhanced recovery of carotenoids, chlorophyll-related pigments, phenolic compounds, and antioxidant activity, but it also resulted in higher acid value (AV), peroxide value (PV), *para*-anisidine value (*p*-AnV), Total Oxidation Value (TOTOX), and Integral Oxidation Value (INTOX). Japanese quince seed oils, especially those obtained by Soxhlet and room-temperature hexane extraction, showed the highest oxidative stability, whereas Folch-extracted grape and strawberry seed oils were the least stable. Overall, extraction method affected oil quality and bioactive compound recovery more strongly than fatty acid composition.

Keywords: fruit seed oils; oil extraction; fatty acid composition; bioactive compounds; antioxidant activity; oxidative stability; lipid oxidation; agro-industrial by-products



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1. Introduction

Large amounts of by-products, such as seeds, pomace, peels, and other solid residues, are generated during fruit processing. Although these fractions are often discarded, fruit seeds are increasingly recognized as valuable sources of lipids and bioactive compounds. Their valorization aligns with sustainable food processing and circular economy principles, as it enables the recovery of high-value components from agro-industrial residues. Fruit seed oils are rich in essential fatty acids, particularly unsaturated ones, and contain minor bioactive components such as phytosterols, tocopherols, carotenoids, chlorophylls, phenolics, phospholipids, and other lipophilic substances [1]. These components influence the oils' nutritional and functional qualities, as well as their oxidative stability and suitability for use in the food, nutraceutical, pharmaceutical, and cosmetic industries [2].

The four seed materials analyzed in this study were selected not only for their availability as fruit-processing by-products but also for their distinct compositional and functional characteristics. Rosehip (*Rosa canina* L.), Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach), grape (*Vitis vinifera* L.), and strawberry (*Fragaria × ananassa* (Duchesne ex Weston) Duchesne ex Rozier) seeds differ in expected lipid profiles, particularly in the proportions of polyunsaturated and monounsaturated fatty acids, as well as in the composition of lipid-associated bioactive compounds. Therefore, these materials provide a useful comparative model for evaluating how extraction methods influence oil recovery, physicochemical quality, antioxidant potential, and oxidative stability. Unconventional fruit seed oils, such as those from rosehip, Japanese quince, grape, and strawberry seeds, are especially noteworthy. Rosehip seed oil is one of the most widely discussed non-conventional fruit seed oils. Its nutritional value is largely attributed to the high proportion of polyunsaturated fatty acids, particularly linoleic and α -linolenic acids. However, this composition also makes the oil more susceptible to oxidative deterioration. Besides fatty acids, rosehip seed oil includes sterols, tocopherols, polyphenols, phospholipids, and pigments that enhance its functional properties and explain its increasing use in food, pharmaceutical, and cosmetic sectors. However, the oil's composition and quality can be greatly influenced by the extraction method, as extraction conditions affect both oil yield and the preservation of oxidation-sensitive bioactive compounds [3–5]. Japanese quince seed oil, derived from seeds during the processing of *Chaenomeles japonica* fruit, is less common but especially interesting. Unlike rosehip seed oil, it is mainly high in oleic and linoleic acids, with palmitic acid being the dominant saturated fatty acid. This oil stands out for its rich minor lipophilic fraction, with α -tocopherol as the main tocopherol and β -sitosterol as the primary phytosterol. Studies have also noted the presence of squalene and have indicated that factors such as genotype, cultivar, and harvest year can affect oil yield, fatty acid profile, and concentrations of tocopherols, phytosterols, and squalene. Consequently, Japanese quince seed oil can be regarded not only as a source of unsaturated lipids but also as a product with a distinctive profile of bioactive lipophilic compounds [6]. Grape seed oil is distinct from other oils mainly because it is closely linked to the winemaking industry, where grape seeds are a plentiful by-product. It is well known as a rich source of linoleic acid, with unsaturated fatty acids making up most of its fatty acid content. Besides fatty acids, grape seed oil also contains vitamin E compounds and phytosterols. Phenolic compounds such as gallic acid, catechin, epicatechin, procyanidins, and proanthocyanidins are especially important for their antioxidant and health benefits. Although phenolics are primarily found in grape seeds and extracts rather than in the oil itself, extraction methods can affect how much of these compounds are recovered. Therefore, grape seed oil serves as a useful model for studying how extraction processes influence lipid recovery and the transfer of minor antioxidant compounds into the oil [7]. Strawberry seed oil, a notable berry seed oil, is gaining attention due to growing interest in berry-processing by-products.

Although it has been less studied than grape seed oil, existing data suggest that it is high in unsaturated fatty acids, particularly linoleic acid, and contains a significant amount of α -linolenic acid. Recent research indicates that extraction methods can influence several physicochemical and quality-related traits, such as pigment content, tocopherol profile, antioxidant capacity, thermal behavior, and oxidative stability. However, the fatty acid profile seems less affected by the extraction process compared to other oil characteristics. This makes strawberry seed oil particularly useful for studying whether different extraction techniques primarily alter minor bioactive components and oxidative stability, rather than the core fatty acid composition [8].

Given the compositional diversity and varying technological potentials of oils from rosehip, Japanese quince, grape, and strawberry seeds, the extraction process plays a crucial role in defining their final quality. It significantly influences the yield, composition, and physicochemical properties of these oils. Variations in factors such as solvent polarity, extraction temperature, extraction duration, oxygen exposure, and sample preparation can affect lipid and bioactive compound recovery, as well as the formation or removal of oxidation products [2,9].

Conventional solvent-based methods, such as Soxhlet extraction, are widely used for their efficiency, but they may require higher temperatures and longer extraction times, which can affect oil quality and the stability of sensitive compounds. In contrast, cold solvent extraction may reduce thermal degradation, while methods based on chloroform–methanol systems, such as the Folch method, may improve the recovery of more polar lipid fractions. In this context, the Folch method was included not as a model for industrial oil production but as an analytical reference method to compare the broader recoverable lipid fraction and lipid-associated bioactive compounds with oils obtained using hexane-based procedures. Therefore, comparative studies are needed to understand how different extraction procedures affect not only oil yield, but also physicochemical properties, fatty acid composition, bioactive compound content, and oxidative stability.

This study aimed to compare how Soxhlet extraction, room-temperature hexane extraction, and the Folch method affect the yield, physicochemical properties, fatty acid profile, nutritional lipid indicators, bioactive compounds, antioxidant activity, and oxidative stability of oils from rosehip, Japanese quince, grape, and strawberry seeds. Using a consistent analytical approach across these four types of seed oils, the research intended to evaluate how extraction methods influence oil quality and to explore the main relationships between extraction efficiency, oxidation levels, lipid composition, bioactive components, and oxidative stability.

2. Materials and Methods

2.1. Materials, Reagents, and Seed Preparation

All reagents, solvents, and analytical standards used in this study were of analytical grade, unless specified otherwise. They were obtained from Merck Life Science Sp. z o.o. (Poznań, Poland) and Avantor Performance Materials Poland S.A. (Gliwice, Poland). The main chemicals and reagents used in the experiments included: *n*-hexane ($\geq 95\%$), ethanol (96% , *v/v*), methanol ($\geq 99.8\%$), chloroform ($\geq 99\%$), isooctane ($\geq 99\%$), toluene ($\geq 99.5\%$), glacial acetic acid ($\geq 99.8\%$), potassium hydroxide (KOH), potassium iodide (KI), sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$; $\geq 99\%$), anhydrous magnesium sulfate (MgSO_4 ; $\geq 99\%$), *p*-anisidine, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3 ; $\geq 99.5\%$), DPPH ($\geq 95\%$), ABTS ($\geq 98\%$), Trolox ($\geq 97\%$), and ammonium persulfate ($\geq 98\%$).

The study material consisted of rosehip (*R. canina* L.; R), Japanese quince (*C. japonica* (Thunb.) Lindl. ex Spach; JQ), grape (*V. vinifera* L.; G), and strawberry (*F. × ananassa* (Duchesne ex Weston) Duchesne ex Rozier; S) seeds purchased from Polish suppliers via online

retail platforms. Grape seeds were obtained from the 2025 harvest at a vineyard in Wołów, near the Jezierzycza Landscape Park, Lower Silesian Voivodeship, Poland. According to the supplier's declaration, the material originated from mixed grape cultivars/varieties, including Frankenthaler, Gołubok, Green Moldavian, Seyve Villard, and Cabernet Sauvignon. Japanese quince seeds (*C. japonica*) were obtained from fruits collected in 2025 from a private garden in Lubaczów, Podkarpackie Voivodeship, Poland. Rosehip seeds were obtained from dried wild rosehip fruits collected in the Bieszczady Mountains, Podkarpackie Voivodeship, Poland, in 2025. Strawberry seeds were purchased from RAFEX (Ciecierzyn, Lublin Voivodeship, Poland).

2.2. Oil Extraction Procedures

Before extraction, all seed materials were visually inspected and, when necessary, cleaned of visible impurities. The seeds were ground immediately before extraction using an electric grinder (Esperanza, Ożarów Mazowiecki, Poland) to obtain a homogeneous material and improve solvent penetration. The prepared samples were used for oil extraction by Soxhlet extraction (Sox), room-temperature hexane extraction (RTHE), and the Folch method (F). A unified extraction time of 4 h was used for all three extraction procedures to enable direct comparison of the methods under standardized conditions. The purpose of this study was not to optimize each extraction method individually, but to evaluate how different solvent systems and extraction approaches affect oil yield and quality parameters when extraction duration is held constant.

2.2.1. Soxhlet Extraction

Soxhlet extraction was performed using *n*-hexane as the extraction solvent. Briefly, 20 g of ground seed material was wrapped in filter paper, placed in an extraction thimble, and extracted with 200 mL of *n*-hexane for 4 h at the solvent's boiling point. After extraction, the hexane–oil solution was collected, and the solvent was removed under reduced pressure at 40 °C using an IKA RV 3 eco rotary evaporator (IKA-Werke GmbH & Co. KG, Staufen, Germany). Residual solvent was removed by purging the oil samples with a stream of nitrogen, and the crude oil was weighed and used for further analysis.

2.2.2. Room-Temperature Hexane Extraction

Room-temperature hexane extraction was performed at room temperature. Ground seed material (20 g) were transferred to a flask and combined with 200 mL of *n*-hexane. The suspension was then mechanically agitated for 4 h using a laboratory shaker (IKA KS 4000 ic control, IKA, Staufen, Germany). After extraction, the mixture was filtered through filter paper, and the supernatant was collected. The extract was dried over anhydrous magnesium sulfate to remove residual moisture, then filtered to remove the drying agent. Hexane was evaporated under reduced pressure at 40 °C using a rotary evaporator, and the remaining solvent was removed under a stream of nitrogen. The recovered oil was weighed and stored until analysis.

2.2.3. Folch Extraction

Oil extraction was also performed using the Folch method with slight modifications. Ground seed material (20 g) was mixed with 200 mL of a chloroform/methanol solution (2:1, *v/v*) and shaken for 4 h on a laboratory shaker. The solid residue was collected by filtration. Subsequently, 40 mL of aqueous potassium chloride solution was added to the filtrate, and the mixture was left overnight at 4 °C to allow phase separation. The next day, the biphasic system was allowed to reach room temperature and then transferred to a separatory funnel. The lower chloroform phase containing lipids was collected, dried over anhydrous magnesium sulfate, and filtered. The solvent was evaporated under reduced

pressure, and residual solvent was removed by nitrogen purging. The obtained oil was weighed and stored for further analysis.

2.3. Determination of Oil Extraction Yield

Oil extraction yield was calculated using the mass of oil recovered after solvent evaporation and the initial mass of ground seed material used for extraction, according to the following equation:

$$\text{Oil extraction yield(\%)} = \frac{m_o}{m_s} \times 100 \quad (1)$$

where m_o is the mass of the extracted oil and m_s is the mass of the raw material.

2.4. Determination of Oil Quality Parameters

The physicochemical quality of the extracted oils was evaluated based on standard lipid quality parameters, including acid value (AV), peroxide value (PV), *p*-anisidine value (*p*-AnV), total oxidation value (TOTOX), and integrated oxidation value (INTOX).

2.4.1. Acid Value

The acid value (AV), which indicates the extent of hydrolytic degradation in the oil, was measured as described in the previous study [10]. In short, 2.0 g of oil was placed in a titration vessel and dissolved in 70 mL of a toluene–ethanol (1:1, *v/v*) mixture. The sample was then titrated with 0.1 M potassium hydroxide (KOH) using an automatic titrator (TitraLab AT1000 Series, Hach Lange, Wrocław, Poland). The results were expressed as mg KOH per gram of oil.

2.4.2. Peroxide Value

The peroxide value (PV), which indicates the content of primary lipid oxidation products, was determined according to the AOCS Official Method Cd 8b-90 [11] using the same automated titration system. Briefly, 2.0 g of oil was mixed with 25 mL of a chloroform–acetic acid solution (2:3, *v/v*), after which 1 mL of saturated potassium iodide was added. The sample was shaken for 30 s and incubated in darkness for 5 min. Subsequently, 75 mL of distilled water was added, and the released iodine was titrated with 0.002 M sodium thiosulfate. The PV was reported as mEq O₂/kg oil.

2.4.3. *p*-Anisidine Value

The *p*-anisidine value (*p*-AnV) is used to assess the level of secondary lipid oxidation products [12]. The oil sample was dissolved in isooctane, and the absorbance of the solution was measured at 350 nm using a UV–Vis spectrophotometer (Rayleigh UV1601, Beijing Beifen-Ruili Analytical Instrument (Group) Co., Ltd., Beijing, China). Then, *p*-anisidine reagent was added, and after the reaction, the absorbance was measured again at the same wavelength, and the *p*-AnV was calculated.

2.4.4. TOTOX and INTOX Values

The total oxidation value (TOTOX), which combines primary and secondary oxidation products, was calculated from PV and *p*-AnV using the following equation:

$$\text{TOTOX} = 2 \times \text{PV} + p - \text{AnV} \quad (2)$$

The integrated oxidation value (INTOX) [13], which places greater weight on secondary oxidation products, was calculated using the following equation:

$$INTOX = PV + 2 \times p - AnV \quad (3)$$

where PV is the peroxide value, expressed in mEq O₂/kg of oil, and *p*-AnV is the *p*-anisidine value.

2.5. Fatty Acid Composition and Nutritional Lipid Indices

The fatty acid profile of the oils was determined using gas chromatography with flame ionization detection (GC-FID) after conversion of fatty acids to their methyl ester derivatives. For fatty acid methyl ester (FAME) preparation, a small portion of oil was dissolved in 2 mL of *n*-hexane and reacted with 2 mL of 1 mol/L methanolic KOH. The sample was vortexed, incubated at 40 °C for 20 min, and then allowed to separate into phases. The upper *n*-hexane layer, containing the FAME fraction, was collected, dried with anhydrous magnesium sulfate, and used for chromatographic analysis.

GC-FID analysis was performed using a YL6100 GC system (Young Lin Instrument Co., Anyang, Republic of Korea) equipped with a flame ionization detector and a BPX-70 capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness; SGE Analytical Science, Milton Keynes, UK). Nitrogen (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. Samples were injected in split mode at a split ratio of 1:50. The oven temperature program was as follows: 70 °C for 0.5 min, increased to 160 °C at 15 °C/min, then increased to 200 °C at 1.1 °C/min and held for 12 min, followed by an increase to 225 °C at 30 °C/min with a final hold of 1 min. The injector and detector temperatures were set at 225 °C and 250 °C, respectively.

Fatty acids were identified by comparing their chromatographic retention times with those of the certified Supelco 37 Component FAME Mix reference standard (Sigma-Aldrich, Bellefonte, PA, USA). Relative amounts were calculated using peak-area normalization and reported as the percentage of each fatty acid in the total pool of identified fatty acids. Measurements were performed in duplicate.

Based on these compositional data, nutritional lipid quality indices were calculated, including AI (atherogenicity index), TI (thrombogenicity index), h/H ratio (hypocholesterolemic/hypercholesterolemic fatty acid ratio), *n*-6/*n*-3 ratio, and PUFA/SFA ratio. The indices were calculated according to the following equations [10,14]:

$$AI = \frac{C12 : 0 + (4 \times C14 : 0) + C16 : 0}{\sum MUFA + \sum n - 6PUFA + \sum n - 3 PUFA} \quad (4)$$

$$TI = \frac{C14 : 0 + C16 : 0 + C18 : 0}{0.5 \times \sum MUFA + (0.5 \times \sum n - 6 PUFA) + (3 \times \sum n - 3 PUFA) + \frac{\sum n - 3 PUFA}{\sum n - 6 PUFA}} \quad (5)$$

$$h/H = \frac{C18 : 1 + \sum PUFA}{C12 : 0 + C14 : 0 + C16 : 0} \quad (6)$$

2.6. Determination of Carotenoid and Chlorophyll Contents

2.6.1. Determination of Total Carotenoid Content

Total carotenoid content was determined spectrophotometrically at 430 nm and expressed as mg β-carotene equivalents per kg of oil. Oil samples of known mass (0.1 g) were diluted to a final volume of 10 mL with isooctane as the solvent, and absorbance was measured at 430 nm against a solvent blank. Quantification was performed using a β-carotene calibration curve ($A_{430} = 149.32C - 0.0098$, $R^2 = 0.9966$), where A_{430} is the absorbance at 430 nm, and C is the β-carotene concentration in the analyzed solution. The total carotenoid content was calculated using the following equation:

$$\text{Total carotenoid content (mg } \beta \text{ - carotene equivalents/kg oil)} = \frac{\left(\frac{A_{430} + 0.0098}{149.32} \right) \times V \times \text{DF} \times 1000}{m} \quad (7)$$

where V is the final volume of the sample solution (mL), m is the oil mass used for analysis (g), and DF is the dilution factor.

2.6.2. Determination of Chlorophyll-Related Pigments

Chlorophyll-related pigments were quantified by UV–Vis spectrophotometry using the standardized 630/670/710 nm method of Pokorný et al. [15], with a modification to account for sample dilution. Briefly, an accurately weighed amount of oil (0.1 g) was diluted with isooctane to a known final volume (25 mL) and thoroughly mixed. The absorbance of the resulting solution was measured at 630, 670, and 710 nm against isooctane as the blank in a 1 cm cuvette. The content of chlorophyll-related pigments was expressed as mg pheophytin a equivalents per kg of oil.

The chlorophyll-related pigment content was calculated as follows:

$$\text{Chlorophyll – related pigments (mg pheophytin a/kg oil)} = \frac{\left(A_{670} - \frac{(A_{630} - A_{710})}{2} \right) \times V}{0.0964 \times L \times m} \quad (8)$$

where A_{630} , A_{670} , and A_{710} are the absorbance values at 630, 670, and 710 nm, respectively; V is the final volume of the diluted sample (mL); m is the mass of oil used for analysis (g); and L is the optical path length of the cuvette (cm). For a 1 cm cuvette, $L = 1$. This calculation is a dilution-corrected adaptation of the standard spectrophotometric procedure, which quantifies chlorophyll pigments in oils as pheophytin a equivalents.

2.7. Determination of Bioactive Compounds and Antioxidant Activity

Oil extracts for determining total phenolic content and antioxidant activity were prepared using 80% methanol. Briefly, 0.5 g of oil was mixed with 2.5 mL of *n*-hexane and 2.5 mL of 80% methanol. The mixture was shaken for 2 min and then centrifuged at 8000 rpm for 10 min. After centrifugation, the lower hydroalcoholic layer was collected in a separate tube. The extraction procedure was repeated three times, and the collected 80% methanolic extracts were combined and used for further analyses. The extraction was performed according to the procedure described by Siol et al. [16], with minor modifications.

2.7.1. Total Phenolic Content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method [17]. For the assay, 0.18 mL of 80% methanolic oil extract was mixed with 4.92 mL of distilled water and 0.30 mL of Folin–Ciocalteu reagent. After 3 min, 0.60 mL of 17.7% (*w/v*) sodium carbonate solution was added. The mixture was vortexed and incubated in the dark for 60 min. Absorbance was measured at 750 nm against a reagent blank. The results were calculated using a gallic acid calibration curve and expressed as mg gallic acid equivalents per gram of oil (mg GAE/g oil).

2.7.2. DPPH Radical Scavenging Activity

DPPH radical-scavenging activity was determined using a methanolic DPPH solution [18]. Briefly, 0.30 mL of the 80% methanolic oil extract was mixed with 2.70 mL of 0.004% (*w/v*) DPPH solution. The mixture was vortexed and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm against a reagent blank. Antioxidant activity was calculated from a Trolox calibration curve and expressed as μmol Trolox equivalents per gram of oil ($\mu\text{mol TE/g oil}$).

2.7.3. ABTS Radical Cation Scavenging Activity

ABTS radical cation-scavenging activity was determined using the ABTS⁺ assay [19]. The ABTS radical cation solution was prepared by mixing 7 mM ABTS with ammonium persulfate to achieve a final ammonium persulfate concentration of 2.45 mM. The mixture was incubated in the dark at room temperature for 16 h to generate ABTS⁺. Before analysis, the ABTS⁺ working solution was diluted with distilled water to an absorbance of 0.700 ± 0.020 at 734 nm. For the assay, 40 μ L of the 80% methanolic oil extract was mixed with 4.0 mL of the ABTS⁺ working solution. After 6 min of incubation, absorbance was measured at 734 nm against a reagent blank. Antioxidant activity was calculated using a Trolox calibration curve and expressed as μ mol Trolox equivalents per gram of oil (μ mol TE/g oil).

2.8. Oxidative Stability Analysis

The oxidative stability of the extracted oils was measured by isothermal pressure differential scanning calorimetry (PDSC) on a DSC Q20P calorimeter (TA Instruments, New Castle, DE, USA). Oil samples weighing approximately 3–4 mg were placed in open aluminum pans, and an empty open aluminum pan served as the reference. The samples were analyzed under oxidative conditions at 120 °C and an oxygen pressure of 1350–1400 kPa. The oxidation onset time (τ_{on}) and the time to reach the maximum oxidation rate (τ_{max}), determined from the heat flow curve, were recorded as indicators of oil oxidative stability.

2.9. Statistical Analysis

Unless indicated otherwise, measurements were carried out in triplicate, and the data were reported as mean values with standard deviations. Statistical analysis was performed using Statistica 13.3 software (TIBCO Software Inc., Palo Alto, CA, USA). Differences among samples were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at a significance level of $p < 0.05$. Pearson correlation analysis was used to assess relationships among the analyzed parameters. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed to evaluate similarities and differences among oils obtained from different seed materials and extraction methods.

3. Results and Discussion

The first stage of the analysis focused on oil extraction yield, which enabled comparison of lipid recovery efficiency across different seed materials and extraction methods. This feature varied considerably depending on the seed material and, to a lesser extent, on the extraction procedure used (Figure 1). Overall, the extraction yields ranged from 4.34% to 10.76%, indicating marked differences in the amount of recoverable lipid fraction among the analyzed fruit seeds.

The lowest oil yields were obtained from rosehip seeds, regardless of the extraction method. The values for R-Sox, R-RTHE, and R-F were $4.85 \pm 0.03\%$, $4.34 \pm 0.08\%$, and $4.56 \pm 0.29\%$, respectively. In contrast, grape, Japanese quince, and strawberry seeds generally showed higher oil recovery. The highest yields were observed for JQ-Sox and G-RTHE, reaching $10.76 \pm 0.74\%$ and $10.76 \pm 0.11\%$, respectively. Similarly high values were recorded for G-Sox ($10.42 \pm 0.48\%$) and S-F ($10.36 \pm 0.06\%$). For strawberry seeds, the Folch method yielded the highest yield, whereas room-temperature hexane extraction gave the lowest among the strawberry samples. For grape seeds, all three extraction methods produced relatively high and comparable oil yields. Then, a different trend was observed for Japanese quince seeds: Soxhlet extraction yielded the highest yield, whereas the Folch method resulted in a markedly lower oil recovery of $7.48 \pm 0.88\%$. These results indicate

that the effect of extraction method was dependent on the type of seed material, with rosehip seeds showing consistently low oil recovery and grape seeds showing consistently high recovery across all extraction procedures.

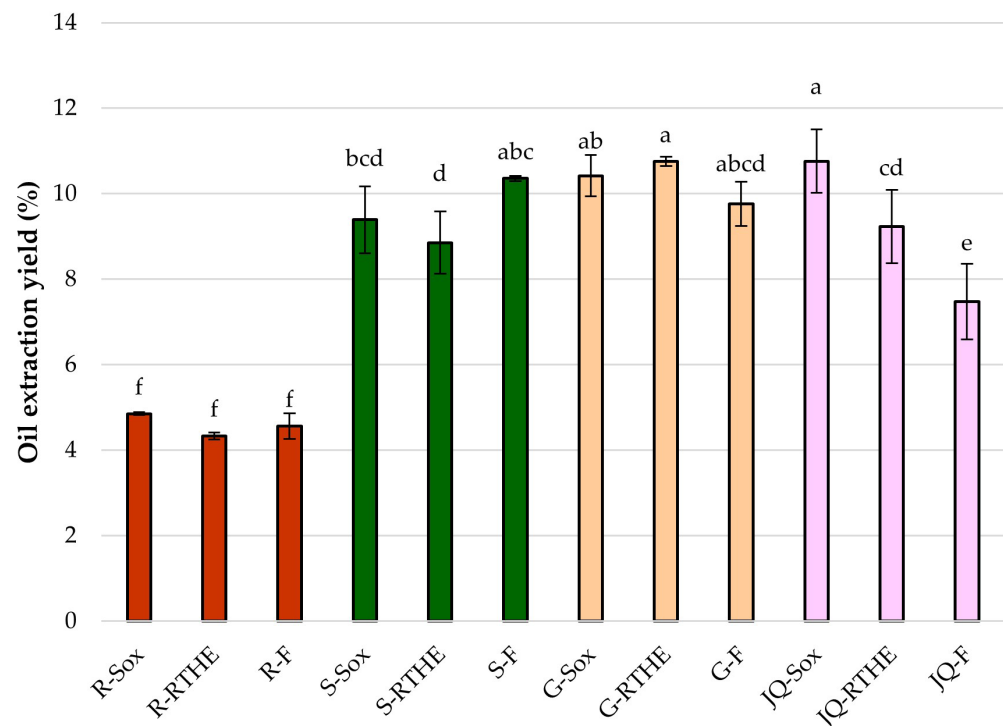


Figure 1. Oil extraction yield (%) of the analyzed samples. Values are presented as mean $\pm$ standard deviation. Different lowercase letters above the bars indicate statistically significant differences between samples according to Tukey's test at $p < 0.05$. Abbreviations are defined in the text.

The oil yields in the present study were generally consistent with the broad ranges reported for unconventional fruit seed oils, though clear differences were observed across seed materials. The relatively low yield of rosehip seed oil, ranging from 4.34% to 4.85%, aligns with literature data indicating that rosehip seeds may contain approximately 1.31–16.50% oil, depending on the extraction technique, sample type, solvent, particle size, and extraction conditions [3]. In previous studies, Soxhlet extraction of rosehip seed oil yielded approximately 6.49–7.76%, while hexane extraction yielded around 4.85%, values close to those obtained in the present study [3]. The yields for JQ-Sox and JQ-RTHE, at 10.76% and 9.23%, respectively, were near the lower end of the reported range for Japanese quince seed oil. Mišina et al. [6] reported oil yields of 10.9–14.6% dry weight for twelve Japanese quince genotypes, with an average of 12.8% [6]. The lower yield observed for JQ-F in the present study may suggest that the Folch method, under the conditions used, was less effective than hexane-based extraction in recovering the neutral lipid fraction from Japanese quince seeds.

The grape seed oil yields in this study, ranging from 9.76% to 10.76%, were consistent with previously reported values. Grape seeds generally contain 6–20% oil, with the final yield depending on grape variety, seed maturity, environmental conditions, and the extraction protocol [7]. The report also indicated that the composition and yield of grape seed oil depend mainly on raw material characteristics, while the extraction protocol plays a secondary role [7]. For strawberry seed oil, extraction yields were relatively high, particularly with the Folch method. Recent studies on berry seed oils indicate that strawberry seeds may yield more oil than raspberry and blackcurrant seeds, depending on the extraction method [8]. Rajagukguk et al. [8] reported that supercritical CO₂ extraction produced the

highest oil yields from berry seeds, whereas *n*-hexane extraction and cold pressing yielded less. Importantly, the authors emphasized that factors such as seed dryness, particle size, and temperature strongly affect extraction efficiency [8]. Therefore, the relatively high yield observed for S-F in the present study may be related not only to solvent polarity but also to improved recovery of lipid fractions using the chloroform-methanol system.

The oxidative quality of the extracted oils was assessed using parameters that describe hydrolytic degradation, primary oxidation products, and secondary oxidation products. Clear differences were observed across both seed materials and extraction methods (Figure 2, Table 1).

The acid value (AV) ranged from 1.84 ± 0.98 to 17.60 ± 1.37 mg KOH/g oil. The highest AV was observed for S-F, indicating the greatest extent of hydrolytic degradation among the oils analyzed. High AVs were also observed for S-RTHE, S-Sox, and R-F, whereas the lowest values were found for JQ-RTHE, G-Sox, JQ-Sox, G-RTHE, R-Sox, and R-RTHE. In general, oils obtained using the Folch method showed higher AVs than those obtained by Soxhlet and room-temperature hexane extraction.

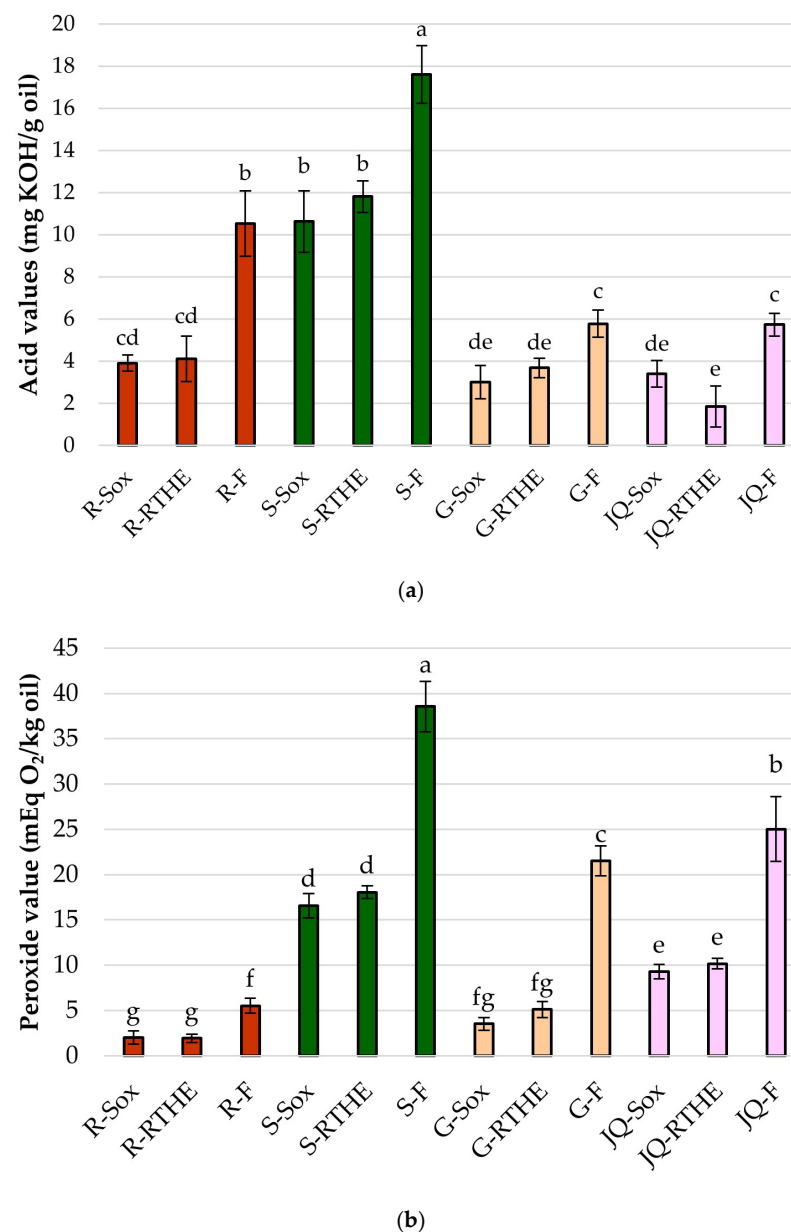


Figure 2. Cont.

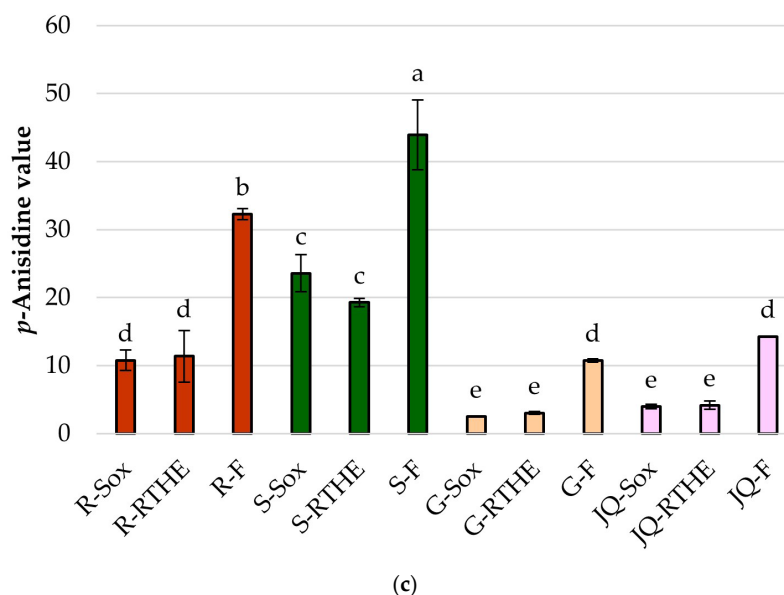


Figure 2. Oxidation quality parameters of oils extracted from the analyzed samples: (a) acid value (mg KOH/g oil), (b) peroxide value (mEq O₂/kg oil), and (c) *p*-anisidine value. Values are presented as mean $\pm$ standard deviation. Different lowercase letters above the bars indicate statistically significant differences between samples according to Tukey's test at $p < 0.05$. Abbreviations are defined in the text.

Table 1. Total oxidation value (TOTOX) and integrated oxidation value (INTOX) of oils extracted from the analyzed samples. Values are presented as mean $\pm$ standard deviation. Different lowercase superscript letters within the same column indicate statistically significant differences between samples according to Tukey's test at $p < 0.05$. Abbreviations are defined in the text.

Sample	TOTOX	INTOX
R-Sox	14.76 $\pm$ 0.02 ^{fg}	23.56 $\pm$ 2.24 ^f
R-RTHE	15.24 $\pm$ 2.86 ^{fg}	24.70 $\pm$ 7.09 ^f
R-F	43.30 $\pm$ 0.89 ^d	70.05 $\pm$ 0.77 ^b
S-Sox	56.73 $\pm$ 0.03 ^{bc}	63.71 $\pm$ 4.09 ^{bc}
S-RTHE	55.36 $\pm$ 1.98 ^c	56.59 $\pm$ 1.88 ^{cd}
S-F	121.02 $\pm$ 10.70 ^a	126.39 $\pm$ 13.02 ^a
G-Sox	9.53 $\pm$ 1.42 ^g	8.52 $\pm$ 0.78 ^h
G-RTHE	13.25 $\pm$ 1.57 ^g	11.19 $\pm$ 0.53 ^{gh}
G-F	53.77 $\pm$ 3.52 ^c	43.02 $\pm$ 2.08 ^e
JQ-Sox	22.56 $\pm$ 1.25 ^{ef}	17.24 $\pm$ 0.11 ^{fgh}
JQ-RTHE	24.51 $\pm$ 0.54 ^e	18.53 $\pm$ 0.62 ^{fg}
JQ-F	64.31 $\pm$ 7.19 ^b	53.55 $\pm$ 3.72 ^d

The peroxide value (PV), reflecting the level of primary oxidation products, ranged from 1.93 ± 0.46 to 38.55 ± 2.79 mEq O₂/kg oil. The lowest PVs were observed for R-RTHE and R-Sox, followed by G-Sox and G-RTHE. In contrast, S-F showed the highest PV, while JQ-F and G-F also exhibited relatively high values. This indicates that the Folch method generally promoted higher recovery or formation of primary oxidation products, especially in strawberry, Japanese quince, and grape seed oils. A similar pattern was observed for the *p*-anisidine value, which ranged from 2.51 ± 0.04 to 43.92 ± 5.11 . The highest *p*-AnV was again recorded for S-F, followed by R-F and S-Sox, indicating greater accumulation of secondary oxidation products in these oils.

The combined oxidation indices confirmed these trends (Table 1). TOTOX values ranged from 9.53 ± 1.42 for G-Sox to 121.02 ± 10.70 for S-F, whereas INTOX values ranged

from 8.52 ± 0.78 to 126.39 ± 13.02 for the same samples. The lowest overall oxidation status was observed for G-Sox, G-RTHE, R-Sox, and R-RTHE, while S-F was clearly the most oxidized oil. In all seed materials, oils obtained by the Folch method showed higher TOTOX and INTOX values than the corresponding oils obtained by Soxhlet or room-temperature hexane extraction. This suggests that the extraction procedure strongly affected the oxidative quality of the recovered oils, with the Folch method yielding oils characterized by higher levels of both primary and secondary oxidation products.

The observed differences in AV, PV, and *p*-AnV indicate that the extraction procedure affected not only the amount of recovered oil but also its hydrolytic and oxidative quality. Previous studies on fruit seed oils, including berry seed oils and grape seed oil, have shown that AV, PV, and *p*-AnV are useful indicators of oil freshness and oxidation status, and that these parameters may be strongly affected by raw material quality, pre-processing conditions, drying, storage, oxygen exposure, and the extraction procedure [20–22]. This is particularly important for oils derived from fruit-processing by-products, such as grape seeds from winemaking or berry seeds from juice and pomace processing, because these materials may be exposed to variable technological and environmental conditions prior to oil extraction.

The influence of the extraction procedure on oil quality indices has also been confirmed in other studies. In almond oil, the extraction method had a stronger effect on quality than the raw material type [10]. The oils obtained by the Folch method had higher levels of primary and secondary oxidation products than those obtained by Soxhlet or cold solvent extraction [10]. Similarly, Yilmaz and Güneşer [23] reported that hexane-extracted lemon seed oil had higher free fatty acid, peroxide, and *p*-anisidine values than cold-pressed oil, despite its higher extraction yield [23]. Bandura et al. [24] also showed that oils obtained by different extraction procedures differed in acid, peroxide, and anisidine values, with microwave-assisted ethanol extraction producing lower values of these indicators than Soxhlet extraction with hexane [24]. These findings support the view that solvent type, solvent polarity, extraction conditions, and sample handling can affect not only the amount of recovered oil but also its hydrolytic and oxidative quality. The high values observed for S-F, R-F, G-F, and JQ-F were also reflected in their TOTOX and INTOX values. Since TOTOX combines PV and *p*-AnV, it provides a broader view of the overall oxidation status, whereas INTOX places greater weight on secondary oxidation products [13]. Therefore, the elevated TOTOX and INTOX values of Folch-extracted oils confirm that these samples were characterized by a higher degree of lipid deterioration involving both primary and secondary oxidation products.

When interpreting the applicability of tested extraction methods, consider the potential presence of residual organic solvents in the recovered oils. In the current study, solvents were removed under reduced pressure and nitrogen purging. However, this does not confirm complete removal of the solvent. For oils used in food, nutraceuticals, or cosmetics, residual solvent analysis, such as headspace GC-FID or GC-MS, should be an extra quality and safety step.

This issue is particularly relevant when comparing hexane-based methods with the Folch method. Hexane is widely used for vegetable oil extraction and may be suitable for technological applications, provided solvent residues are effectively removed and comply with applicable regulatory limits. In the European Union, extraction solvents used in food production are regulated by Directive 2009/32/EC, which sets authorized solvents, conditions of use, and maximum residue limits, including for *n*-hexane in fats and oils [25]. By contrast, the Folch method, which uses a chloroform/methanol mixture, should be considered primarily an analytical or comparative extraction procedure rather than a directly applicable method for producing oils intended for consumption. Although

this solvent system may improve recovery of polar lipids and lipid-associated bioactive compounds, the use of chloroform and methanol raises significant safety concerns and would require extensive purification and analytical verification of residual solvents before any practical application. Thus, the higher recovery of pigments, phenolics, and antioxidant activity observed in Folch-extracted oils should be interpreted primarily as evidence of the extractability of these compounds, rather than as direct evidence of suitability for food-grade oil production.

The fatty acid composition of the analyzed oils is presented in Table 2 and summarized by fatty acid group in Figure 3. Across all samples, polyunsaturated fatty acids (PUFA) were the dominant fraction, followed by monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA). However, the proportions of these groups varied markedly by seed material, whereas the extraction method had only a limited effect on the overall fatty acid profile within the same oil type.

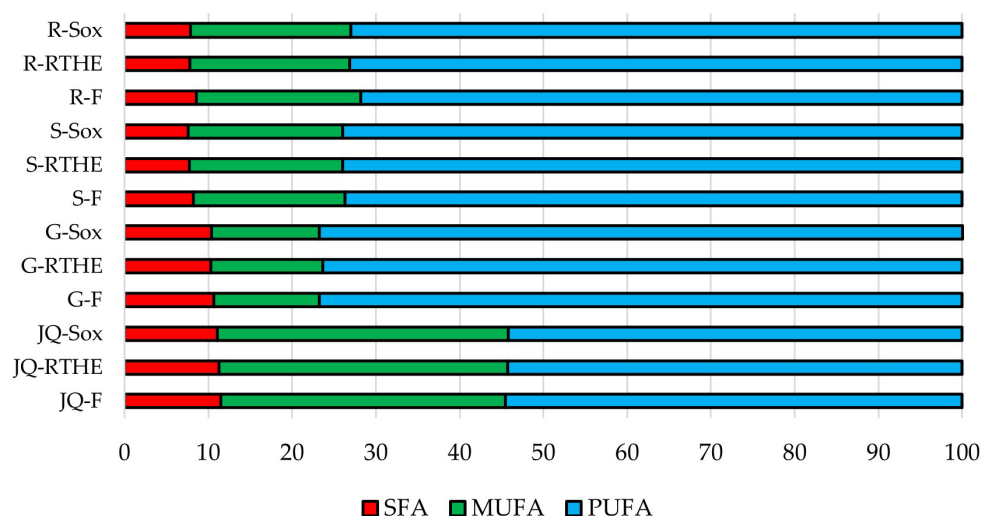


Figure 3. Fatty acid composition of oils extracted from the analyzed samples, expressed as the relative percentage of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Abbreviations are defined in the text.

Grape seed oils had the highest PUFA content, ranging from 76.29% to 76.78%, primarily because of the predominance of linoleic acid (C18:2 *n*-6), which accounted for approximately 75.89–76.28% of total fatty acids. In contrast, α -linolenic acid (C18:3 *n*-3) in grape seed oils was very low, ranging from 0.40% to 0.76%. Strawberry seed oils also had a high PUFA content, reaching 73.68–73.98%, but their fatty acid profile differed from grape seed oils because of the much higher proportion of α -linolenic acid (29.43–29.77%). Rosehip seed oils contained 71.83–73.16% PUFA and were rich in both linoleic acid (52.78–53.31%) and α -linolenic acid (18.74–20.19%). Finally, Japanese quince seed oils had the lowest PUFA content among the analyzed samples, yet PUFA still represented the major fatty acid group, accounting for 54.23–54.51% of total fatty acids.

The MUFA fraction was highest in Japanese quince seed oils, ranging from 34.02% to 34.71%, primarily due to high oleic acid content (33.35–33.97%). In the remaining oils, MUFA levels were lower, ranging from 12.57% to 19.61%. The SFA fraction was generally low across all samples, with the lowest values observed in strawberry and rosehip seed oils. Japanese quince seed oils had the highest SFA content, ranging from 11.07% to 11.47%, mainly due to the relatively high contribution of palmitic acid (C16:0). Overall, the fatty acid profiles indicate that seed material was the primary factor determining oil composition, while Soxhlet extraction, room-temperature hexane extraction, and the Folch method produced oils with relatively similar fatty acid distributions within each seed type.

Table 2. Fatty acid composition and nutritional lipid indices of oils extracted from the analyzed samples. Fatty acids are expressed as relative percentages of total identified fatty acids. Values are presented as mean $\pm$ standard deviation. Different lowercase superscript letters within the same row indicate statistically significant differences between samples according to Tukey's test at $p < 0.05$. ND—not detected; SFA—saturated fatty acids; PUFA—polyunsaturated fatty acids; AI—atherogenicity index; TI—thrombogenicity index; h/H—hypocholesterolemic/hypercholesterolemic fatty acid ratio.

	R-Sox	R-RTHE	R-F	S-Sox	S-RTHE	S-F	G-Sox	G-RTHE	G-F	JQ-Sox	JQ-RTHE	JQ-F
C16:0	3.70 $\pm$ 0.12 ^f	3.84 $\pm$ 0.12 ^{ef}	4.31 $\pm$ 0.03 ^{ef}	4.68 $\pm$ 0.16 ^{cde}	4.90 $\pm$ 0.23 ^{cd}	5.27 $\pm$ 0.50 ^c	6.97 $\pm$ 0.64 ^b	6.72 $\pm$ 0.33 ^b	7.40 $\pm$ 0.10 ^b	9.25 $\pm$ 0.33 ^a	9.41 $\pm$ 0.61 ^a	9.61 $\pm$ 0.83 ^a
C18:0	2.91 $\pm$ 0.04 ^e	2.78 $\pm$ 0.01 ^f	3.12 $\pm$ 0.01 ^d	1.80 $\pm$ 0.06 ^h	1.80 $\pm$ 0.08 ^h	1.97 $\pm$ 0.08 ^g	3.43 $\pm$ 0.13 ^b	3.60 $\pm$ 0.02 ^a	3.26 $\pm$ 0.01 ^c	0.90 $\pm$ 0.04 ⁱ	0.95 $\pm$ 0.01 ⁱ	1.01 $\pm$ 0.08 ⁱ
C18:1	18.74 $\pm$ 0.03 ^d	18.74 $\pm$ 0.04 ^d	19.20 $\pm$ 0.16 ^c	18.10 $\pm$ 0.03 ^e	17.98 $\pm$ 0.04 ^{ef}	17.82 $\pm$ 0.01 ^f	12.84 $\pm$ 0.04 ^h	13.40 $\pm$ 0.05 ^g	12.57 $\pm$ 0.08 ⁱ	33.97 $\pm$ 0.17 ^a	33.78 $\pm$ 0.20 ^a	33.35 $\pm$ 0.28 ^b
C18:2 <i>n</i> -6	52.78 $\pm$ 0.12 ^d	53.31 $\pm$ 0.33 ^{bcd}	53.09 $\pm$ 0.62 ^{cd}	44.32 $\pm$ 0.04 ^e	44.21 $\pm$ 0.04 ^e	44.25 $\pm$ 0.04 ^e	76.28 $\pm$ 0.39 ^a	75.89 $\pm$ 0.43 ^a	76.02 $\pm$ 0.09 ^a	53.53 $\pm$ 0.03 ^{bc}	53.56 $\pm$ 0.27 ^{bc}	53.78 $\pm$ 0.33 ^b
C18:3 <i>n</i> -3	20.19 $\pm$ 0.07 ^b	19.86 $\pm$ 0.16 ^b	18.74 $\pm$ 0.48 ^c	29.66 $\pm$ 0.06 ^a	29.77 $\pm$ 0.04 ^a	29.43 $\pm$ 0.23 ^a	0.50 $\pm$ 0.08 ^{de}	0.40 $\pm$ 0.03 ^e	0.76 $\pm$ 0.10 ^d	0.70 $\pm$ 0.01 ^{de}	0.68 $\pm$ 0.01 ^{de}	0.73 $\pm$ 0.01 ^{de}
C20:0	1.26 $\pm$ 0.06 ^a	1.16 $\pm$ 0.04 ^{ab}	1.13 $\pm$ 0.01 ^{abc}	1.09 $\pm$ 0.04 ^{bcd}	1.01 $\pm$ 0.07 ^{cde}	0.96 $\pm$ 0.13 ^{def}	ND	ND	ND	0.92 $\pm$ 0.04 ^{ef}	0.90 $\pm$ 0.08 ^{ef}	0.86 $\pm$ 0.09 ^f
C20:1	0.43 $\pm$ 0.01 ^c	0.34 $\pm$ 0.04 ^e	0.41 $\pm$ 0.01 ^{cd}	0.35 $\pm$ 0.01 ^{de}	0.33 $\pm$ 0.04 ^e	0.31 $\pm$ 0.02 ^e	ND	ND	ND	0.74 $\pm$ 0.04 ^a	0.72 $\pm$ 0.04 ^{ab}	0.67 $\pm$ 0.05 ^b
AI	0.04	0.04	0.05	0.05	0.05	0.06	0.08	0.07	0.08	0.10	0.11	0.11
TI	0.07	0.07	0.08	0.05	0.06	0.06	0.23	0.22	0.23	0.22	0.22	0.23
h/H	24.83	23.97	21.12	19.68	18.78	17.43	12.89	13.37	12.07	9.54	9.37	9.17
<i>n</i> -6/ <i>n</i> -3	2.61	2.68	2.83	1.49	1.49	1.50	155.33	190.05	100.60	77.02	78.77	73.68
PUFA/SFA	9.28	9.42	8.39	9.78	9.59	8.99	7.39	7.40	7.20	4.90	4.83	4.76

The fatty acid profiles obtained in the present study are consistent with the literature, which indicates that fruit seed oils are generally rich in unsaturated fatty acids, although the proportions of PUFA and MUFA depend mainly on botanical origin rather than the extraction procedure [1,2]. Previous studies on grape, berry, rosehip, and Japanese quince seed oils reported that linoleic, α -linolenic, and oleic acids are usually the dominant unsaturated fatty acids, while palmitic and stearic acids are the main saturated fatty acids [3,4,6–8]. In the present study, PUFA predominated in all oils, particularly due to the high contribution of linoleic acid, whereas Japanese quince seed oil was distinguished by a higher MUFA fraction, mainly oleic acid. The relatively small differences among Soxhlet, room-temperature hexane, and Folch extracts within the same seed type indicate that fatty acid composition was mainly determined by the raw material, while the extraction method had a limited effect on the relative fatty acid profile. Similar observations have been reported for berry and other seed oils, where the extraction procedure affected yield, minor compounds, and quality parameters more strongly than the basic fatty acid composition [8,10,23,24].

The calculated nutritional lipid indices (Table 2) confirmed the favorable fatty acid composition of the analyzed oils. The atherogenicity index (AI) and thrombogenicity index (TI) were low in all samples, ranging from 0.04 to 0.11 and from 0.05 to 0.23, respectively. The h/H ratio was highest in rosehip seed oils (21.12–24.83), followed by strawberry seed oils (17.43–19.68), indicating a high proportion of hypocholesterolemic fatty acids relative to hypercholesterolemic fatty acids. The n -6/ n -3 ratio was particularly favorable in strawberry seed oils (1.49–1.50) and rosehip seed oils (2.61–2.83), whereas grape and Japanese quince seed oils exhibited much higher values due to their very low α -linolenic acid content. The PUFA/SFA ratio was also high in all oils, especially in strawberry and rosehip seed oils, further confirming their nutritional potential.

Pigment content was measured to assess the contribution of lipophilic bioactive compounds to the quality of the extracted oils (Figure 4). Total carotenoid content varied widely across the analyzed oils, ranging from 6.97 ± 0.32 to 348.97 ± 5.11 mg β -carotene equivalents/kg oil. The highest carotenoid content was observed in rosehip seed oils, particularly in R-F, which reached 348.97 ± 5.11 mg β -carotene equivalents/kg oil, followed by R-Sox and R-RTHE at 246.69 ± 11.02 and 184.18 ± 0.56 mg β -carotene equivalents/kg oil, respectively. Strawberry seed oils showed considerably lower values, ranging from 37.80 ± 10.48 to 49.91 ± 3.98 mg β -carotene equivalents/kg oil. Grape and Japanese quince seed oils had the lowest carotenoid contents, though in both cases the Folch method yielded slightly higher values than Soxhlet and room-temperature hexane extraction. Overall, the Folch method produced the highest carotenoid recovery for each seed type.

Chlorophyll content showed a different pattern. The highest value was recorded for S-F, at 100.62 ± 14.08 mg pheophytin a equivalents/kg oil, followed by S-RTHE and S-Sox at 31.72 ± 5.99 and 13.43 ± 2.75 mg pheophytin a equivalents/kg oil, respectively. In contrast, rosehip, grape, and Japanese quince seed oils contained much lower levels of chlorophylls. Among these samples, G-F and JQ-F showed slightly higher chlorophyll contents than the corresponding Soxhlet and room-temperature hexane extracts. These results indicate that pigment composition was strongly dependent on seed type, with rosehip seed oils being the richest source of carotenoids, whereas strawberry seed oils, especially those obtained by the Folch method, contained the highest levels of chlorophyll pigments.

The high carotenoid content observed in rosehip seed oils is consistent with previous studies describing rosehip oil as a particularly rich source of carotenoid pigments. Turan et al. [26] reported a total carotenoid content of 218.8 mg/kg in rosehip seed oil, which is close to the values obtained for R-Sox and lower than that observed for R-F in the present study [26]. The higher carotenoid recovery after Folch extraction may be related to the

ability of mixed organic solvent systems to extract a broader range of lipophilic compounds, including pigments associated with lipid fractions. In contrast, the high chlorophyll content observed in strawberry seed oils, especially S-F, agrees with previous findings on berry seed oils, where strawberry seed oil was characterized by the highest chlorophyll content among the analyzed berry oils [27]. This confirms that pigment composition is strongly matrix-dependent and may vary substantially between fruit seed oils. The presence of chlorophylls in oils is technologically important because, unlike carotenoids, which may contribute to antioxidant protection, chlorophyll pigments can act as pro-oxidants under light exposure and may negatively affect oil stability [28].

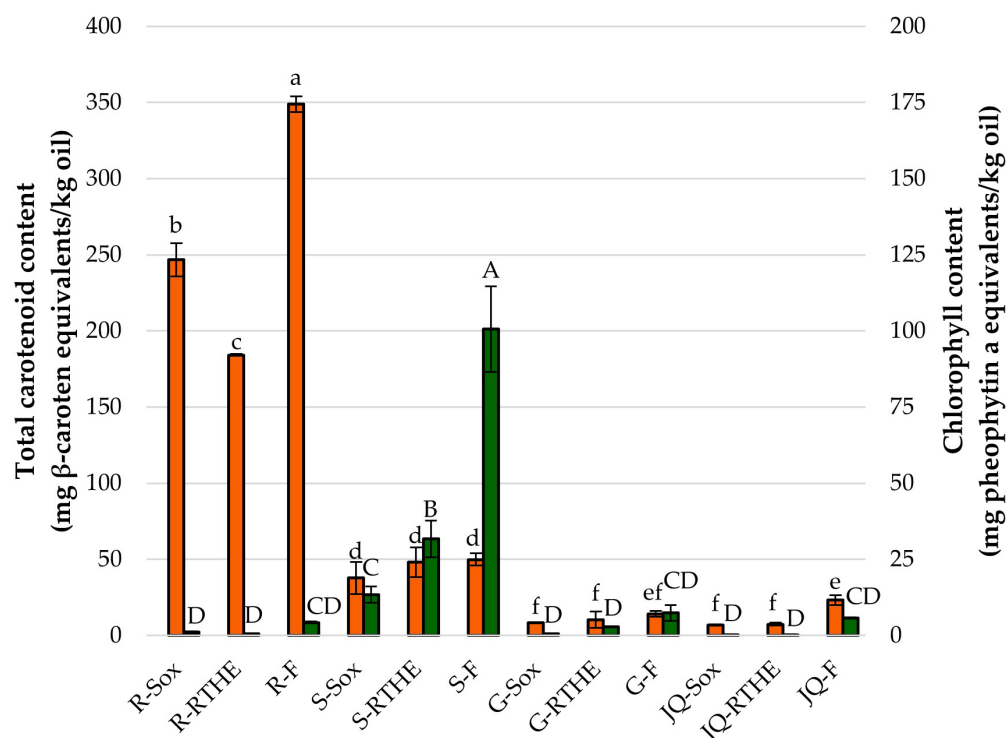


Figure 4. Total carotenoid (orange bars) and chlorophyll (green bars) contents in oils extracted from the analyzed samples. Values are presented as mean $\pm$ standard deviation. Different lowercase letters indicate statistically significant differences between samples for carotenoid content, while different uppercase letters indicate statistically significant differences for chlorophyll content according to Tukey's test at $p < 0.05$. Abbreviations are defined in the text.

The antioxidant activity and total phenolic content were measured to assess the contribution of polar bioactive compounds to the functional properties of the extracted oils (Figure 5). DPPH radical-scavenging activity ranged from 0.38 to 2.84 μmol Trolox equivalents/g oil. The highest DPPH values were recorded for oils obtained by the Folch method, particularly S-F and R-F, at 2.84 and 2.76 μmol TE/g oil, respectively. G-F also showed relatively high DPPH activity, reaching 1.71 μmol TE/g oil, whereas the lowest values were observed for JQ-RTHE, R-RTHE, and JQ-Sox. A similar trend was observed for ABTS antioxidant activity, although the differences among samples were more pronounced. The highest ABTS values were found for S-F and R-F, reaching 13.73 and 12.19 μmol TE/g oil, respectively, followed by G-F and JQ-F. In contrast, the lowest ABTS activity was observed in G-Sox and JQ-Sox.

Total phenolic content (TPC) ranged from 0.16 to 3.10 mg gallic acid equivalents/g oil. The highest TPC was observed in S-F, followed by R-F, G-F, and G-RTHE. In general, Folch-extracted oils showed higher TPC and antioxidant activity than those obtained by Soxhlet or room-temperature hexane extraction. This suggests that the Folch method was

more effective at recovering polar antioxidant compounds, possibly due to the chloroform–methanol solvent system. Overall, strawberry and rosehip seed oils obtained by the Folch method exhibited the highest antioxidant potential among the analyzed samples.

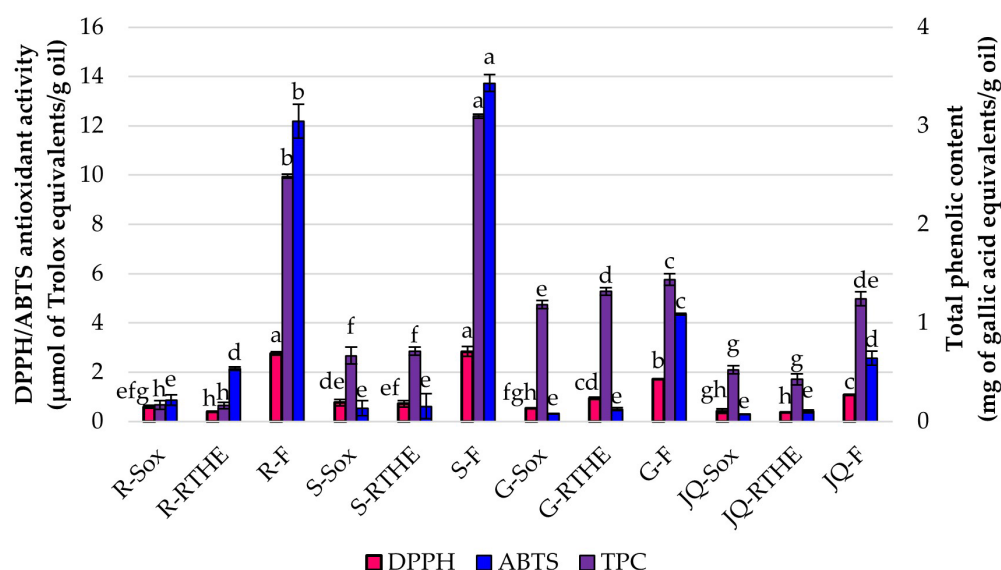


Figure 5. Antioxidant activity and total phenolic content of oils extracted from the analyzed samples. DPPH and ABTS radical-scavenging activities are expressed as $\mu\text{mol TE/g oil}$ and presented on the left y-axis, whereas TPC is expressed as mg GAE/g oil and presented on the right y-axis. Values are presented as mean $\pm$ standard deviation. Different lowercase letters above the bars indicate statistically significant differences between samples within the same parameter according to Tukey's test at $p < 0.05$. Abbreviations are defined in the text.

The variations in TPC, DPPH, and ABTS values suggest that the extraction method affected the recovery of antioxidant compounds. In this study, oils extracted using the Folch method generally had higher total phenolic content and exhibited stronger antioxidant activity than those obtained by Soxhlet or cold-hexane extraction, particularly in strawberry and rosehip seed oils. This likely relates to the chloroform–methanol system, which can extract more polar or amphiphilic compounds than nonpolar hexane. Similar findings have been reported for other seed oils: solvent-extracted grape seed oils had higher phenolic content and DPPH activity than cold-pressed oils, while research on plum seed oil indicated that the extraction method influenced phenolic levels and antioxidant activity measured by DPPH and ABTS assays [21,29]. Additionally, DPPH and ABTS results do not always align perfectly because these assays differ in reaction mechanisms, solvent environments, and sensitivity to various antioxidants [30]. Thus, the higher antioxidant activity in Folch-extracted oils likely reflects a broader recovery of antioxidant-active minor compounds rather than solely an increase in phenolics.

Oxidative stability was evaluated using isothermal PDSC based on the oxidation onset time (τ_{on}) and the time to maximum oxidation rate (τ_{max}) (Table 3). The oxidative stability of the analyzed oils varied significantly with seed material and extraction method. The highest stability was observed in Japanese quince seed oil, particularly JQ-Sox, which showed the longest τ_{on} and τ_{max} values, reaching 70.79 ± 3.56 min and 77.95 ± 5.41 min, respectively. JQ-RTHE also exhibited high oxidative stability, with τ_{on} of 51.41 ± 2.60 min and τ_{max} of 60.07 ± 6.34 min. In contrast, the lowest stability was recorded for G-F and S-F, with τ_{on} values of 2.07 ± 0.13 min and 2.20 ± 0.17 min, respectively, and τ_{max} values below 7 min.

For rosehip and grape seed oils, Soxhlet and room-temperature hexane extraction yielded oils with similar oxidative stability. R-Sox and R-RTHE showed τ_{on} values

of 33.14 ± 1.68 and 32.30 ± 1.85 min, respectively, while G-Sox and G-RTHE reached 37.02 ± 3.24 and 32.42 ± 1.12 min, respectively. However, oils obtained using the Folch method had markedly lower oxidative stability across all seed types. This effect was especially pronounced for grape and strawberry seed oils, where G-F and S-F showed the shortest induction times among all samples. Overall, the results indicate that the Folch method, despite providing higher recovery of some bioactive compounds, produced oils with lower oxidative stability, most likely due to their higher oxidation status and/or the co-extraction of compounds that affect oxidation behavior.

Table 3. Oxidative stability parameters of oils extracted from the analyzed samples, expressed as oxidation induction time (τ_{on}) and time to maximum oxidation rate (τ_{max}). Values are presented as mean $\pm$ standard deviation. Different lowercase superscript letters within the same column indicate statistically significant differences between samples according to Tukey's test at $p < 0.05$. Abbreviations are defined in the text.

Sample	τ_{on} (min)	τ_{max} (min)
R-Sox	33.14 ± 1.68 ^{cd}	39.05 ± 1.14 ^d
R-RTHE	32.30 ± 1.85 ^d	37.96 ± 1.68 ^d
R-F	9.25 ± 0.69 ^g	15.00 ± 0.38 ^f
S-Sox	17.40 ± 1.52 ^f	22.21 ± 1.67 ^e
S-RTHE	8.79 ± 1.10 ^g	13.48 ± 1.17 ^f
S-F	2.20 ± 0.17 ^h	6.33 ± 0.16 ^g
G-Sox	37.02 ± 3.24 ^c	43.62 ± 4.94 ^c
G-RTHE	32.42 ± 1.12 ^d	37.81 ± 0.52 ^{cd}
G-F	2.07 ± 0.13 ^h	4.80 ± 0.42 ^g
JQ-Sox	70.79 ± 3.56 ^a	77.95 ± 5.41 ^a
JQ-RTHE	51.41 ± 2.60 ^b	60.07 ± 6.34 ^b
JQ-F	26.89 ± 1.09 ^e	33.20 ± 1.88 ^d

The high oxidative stability of Japanese quince seed oils may be related to their relatively lower PUFA content, higher MUFA fraction, and lower oxidation indices compared with other oils. This interpretation aligns with previous reports indicating that the oxidative stability of seed oils is strongly influenced by fatty acid composition, particularly the relative proportions of MUFA and PUFA [2,12,13,16]. Górska et al. [31] also reported that cranberry seed oil had a much longer oxidation induction time than strawberry and blackcurrant seed oils, which was attributed to its higher oleic acid content and lower PUFA level. In the same study, strawberry seed oil was the least stable sample in PDSC analysis, confirming that oils rich in polyunsaturated fatty acids are generally more susceptible to accelerated oxidation [31].

In the current study, oils extracted using the Folch method exhibited markedly lower oxidative stability across all seed types. Similar relationships among extraction method, oxidation indices, and PDSC oxidative stability have been reported for almond oil, with Folch-extracted oils exhibiting higher levels of primary and secondary oxidation products and lower oxidative stability than oils obtained by Soxhlet or cold-solvent extraction [10].

Overall, the PDSC results indicate that the oxidative stability of the analyzed oils was determined not only by fatty acid composition but also by their initial oxidation status and the types of compounds recovered during extraction. The lower τ_{on} and τ_{max} values of Folch-extracted oils were consistent with their higher AV, PV, p -AnV, TOTOX, and INTOX values, suggesting that oils with a greater degree of hydrolytic and oxidative deterioration were less resistant to accelerated oxidation. In some samples, particularly S-F, the high chlorophyll content may also have contributed to reduced oxidative stability, as chlorophyll pigments can promote lipid oxidation under oxidative and thermal conditions.

Therefore, although the Folch method enhanced the recovery of some bioactive compounds, it also produced oils with lower oxidative stability, most likely due to the combined effects of higher initial oxidation status, broader recovery of polar lipid fractions, and pigment co-extraction.

Finally, multivariate analysis was conducted to provide a comprehensive overview of relationships among the samples and variables (Figure 6). Hierarchical cluster analysis (Figure 6a) revealed two main clusters. The first cluster included mainly rosehip and strawberry seed oils, whereas the second cluster comprised grape and Japanese quince seed oils. These two groups merged only at the highest linkage distance, indicating clear differences in their overall chemical profiles. Within each sample type, oils obtained by Soxhlet extraction (Sox) and room-temperature hexane extraction (RTHE) were generally grouped very closely, particularly R-Sox with R-RTHE, S-Sox with S-RTHE, G-Sox with G-RTHE, and JQ-Sox with JQ-RTHE. This suggests high similarity between oils obtained using these two extraction procedures. In contrast, oils obtained by the Folch method (F) were usually more distant from the corresponding Sox and CHE samples, indicating that this extraction method had a stronger effect on the overall oil profile.

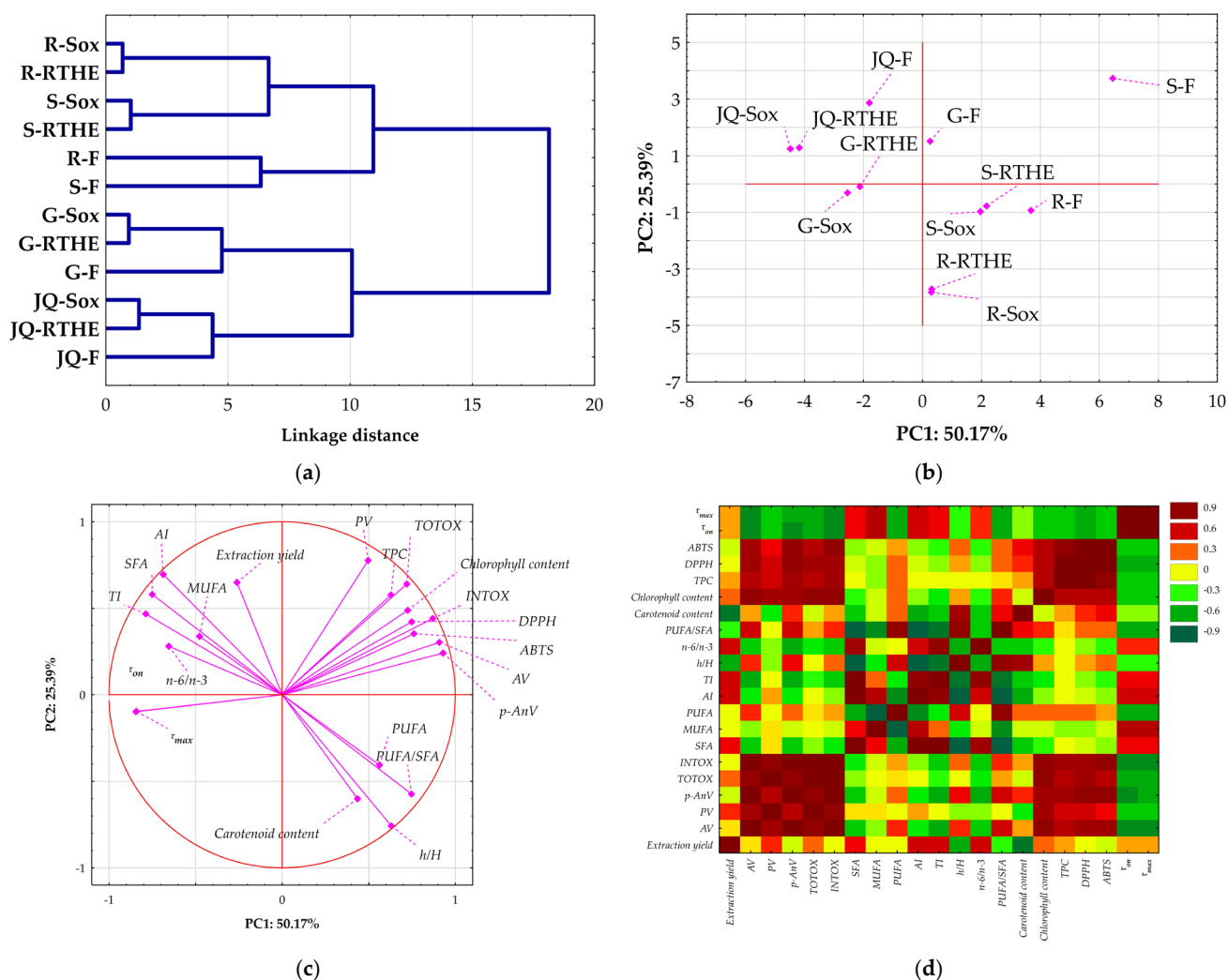


Figure 6. Multivariate analysis of the analyzed oil samples: (a) hierarchical cluster analysis (HCA) dendrogram based on linkage distance, (b) principal component analysis (PCA) score plot showing the distribution of samples on the PC1–PC2 plane, (c) PCA loading plot showing relationships among variables, and (d) correlation heatmap of the analyzed parameters. Abbreviations are defined in the text.

The principal component analysis (PCA) score plot (Figure 6b) confirmed the separation pattern observed in the dendrogram. The first two principal components explained 75.56% of the total variance, with PC1 and PC2 accounting for 50.17% and 25.39%, respectively. The S-F sample was clearly separated from the others and located on the positive side of both PC1 and PC2, indicating its distinct chemical profile. JQ-Sox and JQ-RTHE were strongly positioned on the negative side of PC1, whereas R-Sox and R-RTHE were mainly on the negative side of PC2. The close proximity of oils obtained by Soxhlet and room-temperature hexane extraction within the same sample type further confirmed their similar composition, while oils obtained using the Folch method showed greater displacement in the PCA space.

The PCA loading plot (Figure 6c) indicated that PC1 was primarily associated with lipid oxidation and antioxidant-related parameters. Positive loadings on PC1 were observed for AV, *p*-AnV, TOTOX, INTOX, DPPH, ABTS, TPC, chlorophyll content, and PUFA/SFA, whereas τ_{on} , τ_{max} , SFA, AI, TI, and *n*-6/*n*-3 were located on the opposite side of this component. This distribution suggests that samples with higher oxidation indices and higher antioxidant activity were separated from those with longer oxidative stability times. PC2 was mainly related to PV, TOTOX, extraction yield, AI, and SFA on the positive side, while *h*/*H*, carotenoid content, and PUFA/SFA were positioned on the negative side.

The correlation heatmap (Figure 6d) supported the relationships observed in the PCA loading plot and provided additional insight into the strength of Pearson's correlation coefficients. Strong positive correlations were observed among oxidation indices, particularly between AV and *p*-AnV ($r = 0.945$), AV and INTOX ($r = 0.951$), PV and TOTOX ($r = 0.950$), *p*-AnV and INTOX ($r = 0.966$), and TOTOX and INTOX ($r = 0.950$). In contrast, oxidative stability parameters were negatively correlated with oxidation markers, as shown by the relationships between AV and τ_{on} ($r = -0.736$), AV and τ_{max} ($r = -0.733$), *p*-AnV and τ_{on} ($r = -0.707$), and INTOX and τ_{on} ($r = -0.710$).

Moreover, a strong positive relationship was also observed among antioxidant-related parameters, including TPC and DPPH ($r = 0.939$), TPC and ABTS ($r = 0.891$), and DPPH and ABTS ($r = 0.959$), suggesting that phenolic compounds were closely associated with the antioxidant activity of the extracted oils. In addition, strong correlations between fatty acid composition and nutritional lipid indices, such as SFA with AI ($r = 0.943$), SFA with TI ($r = 0.971$), and PUFA/SFA with AI ($r = -0.966$), were also observed. However, these relationships should be interpreted mainly as a consequence of the mathematical structure of the calculated indices.

Overall, the multivariate analysis confirmed that the oils differed not only in oxidation status and oxidative stability but also in fatty acid composition, pigment content, and antioxidant-related parameters. The combined PCA, HCA, and correlation analysis indicated that the Folch extraction method (F) yielded the most distinct oil profiles, whereas Soxhlet extraction (Sox) and room-temperature hexane extraction (RTHE) generally produced more similar chemical characteristics within the same sample type.

4. Conclusions

Under the standardized comparative conditions used in this study, rosehip, Japanese quince, grape, and strawberry seeds can be valuable by-products for recovering oils rich in unsaturated fatty acids and bioactive compounds. Oil extraction yield was primarily determined by seed type, whereas the influence of the extraction procedure was material-dependent. Rosehip seeds yielded the least oil, while grape, strawberry, and Japanese quince seeds generally showed higher lipid recovery.

The analyzed oils had favorable fatty acid profiles, with a high proportion of unsaturated fatty acids and low atherogenicity and thrombogenicity indices. Fatty acid

composition was mainly determined by botanical origin, whereas Soxhlet extraction, room-temperature hexane extraction, and the Folch method had only a limited effect on the relative fatty acid profile within the same seed type. Rosehip, strawberry, and grape seed oils were dominated by PUFA, whereas Japanese quince seed oils were distinguished by the highest MUFA content, mainly oleic acid.

The extraction method had a much stronger effect on oil quality parameters, pigment content, antioxidant activity, and oxidative stability. Oils obtained by the Folch method generally contained higher levels of carotenoids, chlorophyll-related pigments, and total phenolics, and showed stronger DPPH and ABTS antioxidant activity. However, these oils also had higher AV, PV, *p*-AnV, TOTOX, and INTOX values, indicating greater hydrolytic and oxidative deterioration. This suggests that the chloroform-methanol system recovered a broader lipid fraction, including bioactive compounds as well as compounds associated with oxidation or lower oxidative quality.

PDSC analysis confirmed clear differences in oxidative stability among the analyzed oils. Japanese quince seed oils, especially JQ-Sox and JQ-RTHE, exhibited the highest oxidative stability, likely due to their lower PUFA content, higher MUFA content, and lower oxidation indices. In contrast, Folch-extracted grape and strawberry seed oils exhibited the lowest oxidative stability, consistent with their elevated oxidation indices and, for strawberry seed oil, high chlorophyll content.

Overall, the results indicate that the selection of an extraction method should depend on the intended application of the oil. Under the conditions used in this study, Soxhlet and room-temperature hexane extraction appeared more suitable when oxidative quality and stability were the main priorities, which may be particularly important for potential food, nutraceutical, and cosmetic applications after appropriate residual solvent verification. In contrast, the Folch method may be useful mainly for analytical or comparative purposes when broader recovery of lipid-associated bioactive compounds is required. However, Folch-extracted oils should not be treated as directly applicable food-grade or cosmetic oils without further purification and detailed safety assessment. Therefore, fruit seed oils from agro-industrial by-products have promising potential, but their final quality strongly depends on matching the extraction procedure to the desired technological, nutritional, or functional purpose.

This study also has several limitations. The extraction time was standardized to 4 h to enable direct comparison among methods and was not optimized individually for each seed material or extraction procedure. Residual solvent content was not quantified, and storage stability tests, sensory evaluation, and detailed profiling of individual tocopherols, phytosterols, phenolic compounds, and volatile oxidation products were not included. Future studies should therefore focus on process optimization, residual solvent analysis, scale-up assessment, storage stability evaluation, and detailed compositional profiling to better determine the suitability of fruit seed oils for food, nutraceutical, and cosmetic applications.

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Abbreviations

The following abbreviations are used in this manuscript:

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AI	atherogenicity index
ANOVA	analysis of variance
AV	acid value
DPPH	2,2-diphenyl-1-picrylhydrazyl
G-RTHE	Grape seed oil obtained by room-temperature hexane extraction
G-F	Grape seed oil obtained by Folch extraction
G-Sox	Grape seed oil obtained by Soxhlet extraction
GAE	Gallic acid equivalents
h/H	hypocholesterolemic/hypercholesterolemic fatty acid ratio
HCA	hierarchical cluster analysis
INTOX	integrated oxidation value
JQ-RTHE	Japanese quince seed oil obtained by room-temperature hexane extraction
JQ-F	Japanese quince seed oil obtained by Folch extraction
JQ-Sox	Japanese quince seed oil obtained by Soxhlet extraction
MUFA	monounsaturated fatty acids
ND	not detected
<i>p</i> -AnV	<i>p</i> -anisidine value
PCA	principal component analysis
PDSC	pressure differential scanning calorimetry
PUFA	polyunsaturated fatty acids
PV	peroxide value
R-RTHE	Rosehip seed oil obtained by room-temperature hexane extraction
R-F	Rosehip seed oil obtained by Folch extraction
R-Sox	Rosehip seed oil obtained by Soxhlet extraction
S-RTHE	Strawberry seed oil obtained by room-temperature hexane extraction
S-F	Strawberry seed oil obtained by Folch extraction
S-Sox	Strawberry seed oil obtained by Soxhlet extraction
SFA	saturated fatty acids
TE	Trolox equivalents
TI	thrombogenicity index
TOTOX	total oxidation value
TPC	total phenolic content

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