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Effect of extraction methods on physicochemical and antioxidant properties of oil extracted from plum (*Prunus domestica* L.) seeds

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Abstract. This study investigated the possibility of using plum (*Prunus domestica* L.) seeds, *i.e*. residues of fruit processing, to extract edible oil. Three extraction methods were used in the study: chemical extraction (n-hexane), cold pressing, and supercritical CO_2 extraction. The products obtained using these methods were evaluated in terms of peroxide and acid values, the content of tocopherols, phytosterols and polyphenols, and antioxidant properties (DPPH and ABTS assays). The plum kernel oils were rich in oleic acid. The dominant tocopherol in all variants was δ-tocopherol, with the highest proportion in the SFT (supercritical CO_2 extraction) oil. β-sitosterol and Δ5-avenasterol were the major phytosterols in the oils. The SFT oil had the highest content of total phenols and the strongest antioxidant properties, which was confirmed by the DPPH and ABTS assays. The results suggest that plum kernels can be a potential source of oil for the oil and fat industry. The oil obtained by supercritical CO_2 extraction showed the best fatty acid composition, the highest tocopherol content, and the best antioxidant properties (DPPH and ABTS assays). The use of this type of fruit processing waste contributes to the reduction of waste and supports the circular economy approach.

Keywords: plum kernel oil, extraction, fatty acids, antioxidant properties

1. INTRODUCTION

There are 40 different species of the genus *Prunus*, but only two species are used industrially: the European plum (*Prunus domestica* L.) and the Japanese plum (*Prunus salicina* L.) (Topp *et al.*, 2012). Plums are not only one of the most popular fruits, but also a raw material that is often processed into dried fruit, jams, and juices by the fruit and vegetable industry. In several European countries, plums are also used for the production of alcoholic beverages (Dulf *et al*., 2016). During the processing of these fruits, very large quantities of seeds are produced as waste material, which is why methods for management thereof are being sought. One of these possibilities is the extraction of oil from plum kernels, with the oil content of different varieties ranging from 23% (w/w) to 53% (w/w) on a dry matter basis (Górnaś *et al*., 2017). Veličković *et al*. (2016) identified five main fatty acids, namely oleic, linoleic, palmitic, stearic, and arachidonic acids in plum kernel oil. Due to its high content of unsaturated fatty acids, plum kernel oil is associated with a reduction in LDL cholesterol levels and a possible protective effect against cardiovascular disease (Yu *et al*., 2007). This oil is a valuable source of bioactive compounds, including carotenoids, tocopherols, tocotrienols, phytosterols, and squalene (Savic *et al*., 2020; Bordoni *et al*., 2019). The most important lipophilic compounds in plum kernel oil are β-sitosterol (209-1 259 mg 100 g^{-1} oil) and γ-tocopherol (61-182 mg 100 g^{-1} oil) (Górnaś *et al.,* 2017). Phytosterols contribute to lowering serum cholesterol levels (Chen *et al.,* 2008). Tocopherols and tocotrienols, the biologically active forms of vitamin E, are known for their protective effects against cardiovascular disease and diabetes. Plum kernel oil also contains carotenoids, in particular β-carotene, which exerts provitamin A activity. Another component of plum kernel oil is

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squalene, a biochemical precursor in the biosynthesis of vitamin D, cholesterol, and steroid hormones, all of which play an essential role in maintaining human health (Moreda *et al*., 2001). Various methods can be used to extract the oil fraction from plum kernels. These include three of the most commonly used methods: extraction with organic solvents, extraction with supercritical carbon dioxide, and cold pressing. Although the literature on supercritical CO_2 extraction from plum kernels is not yet extensive, existing studies indicate the great potential of this method. Plum kernel oil obtained in this way is characterised by high quality and high content of valuable bioactive ingredients, which can be used in various industries. The efficiency, quality, and chemical composition of extracted oil depend on the extraction method chosen. Compared to solvent extraction, both cold pressing and supercritical CO_2 extraction yield safer oils and are in line with the concept of "green technologies". The main objective of this study was to evaluate the possibility of producing high-quality plum kernel oil using three different extraction methods: organic solvent extraction, cold pressing, and supercritical CO_2 extraction. This research represents the first direct comparison of three extraction methods: cold pressing, solvent extraction using n-hexane, and supercritical CO_2 extraction used for obtaining oil from plum seeds. The study evaluates their influence on the physicochemical properties, fatty acid profile, tocopherol and phytosterol content, antioxidant capacity, and amygdalin concentration in the resulting oils. The inclusion of toxicological parameters such as amygdalin content adds a distinctive and underexplored dimension to research on fruit seed oils. Notably, the use of supercritical CO_2 extraction conducted in optimised, low-temperature conditions (300 bar, 40°C) as a green technology highlights the innovative aspect of this study, with promising implications for both the food and cosmetic industries.

2. MATERIALS AND METHODS

2.1. Materials

The fully ripe fruits of the plum (*Prunus domestica* L.) cv. 'Diana' were collected near Chełm, Lubelskie Province, Poland. The raw material came from the 2022 harvest. The plum seeds were pretreated according to the procedure described by Rodríguez-Blázquez *et al*. (2023) with minor modifications. The plum seeds were dried for 24 h at 40°C in a Binder Avantgarde Line ED series dryer (Binder, Germany). After drying, the seeds were manually separated into shells and kernels using a hammer. The kernels were ground in an ultra-centrifugal mill (Retsch™ ZM200, Haan, Germany) and sieved through a stainless steel sieve to a particle size < 1 mm. The kernels were stored in tightly sealed transparent zip-lock plastic bags until analysis. The material was stored in a freezer at a temperature of -18°C.

All reagents and chemicals (both analytical and HPLC grade) were provided by Sigma Aldrich (Buchs, Switzerland). Pure standards of phytosterols, tocopherol isomers, and fatty acid methyl esters (FAME) were purchased from Sigma Chemical Co. (St Louis, MO, USA).

2.2. Extraction methods

2.2.1. Oil extraction with n-hexane (OH)

A 50-gram portion of crushed plum kernels was placed into a filter paper thimble and placed into glass Soxhlet apparatus connected to a water condenser and a 500-mL round-bottom flask. The oil extraction was performed with n-hexane (250 mL) in a water bath for 6 hours. After the extraction procedure, the excess solvent was removed under reduced pressure using a rotary evaporator (Hei-VAP Expert Model, Heidolph, Germany) at a temperature of 65°C.

2.2.2. Cold pressing of plum kernel oil (PSO)

Oil was extracted from the kernels using a WT-150 screw oil press (TechnoMaszBud, Poland) with a capacity of 120-150 kg h^{-1}. The process was carried out in the following conditions: a press head temperature of 40°C and frequency of 20 Hz. A nozzle with an internal diameter of 6 mm was used during the extraction.

2.3. Extraction with the use of the SFT system

Supercritical CO_2 Extraction (SFT) was performed with an SFT-120 device (Shimadzu, Japan). The extraction vessel was filled with 100 g of plum kernels. The oil was collected in glass tubes. The extraction time was 5 h. After every 30 min, the extraction process was interrupted and the amount of oil was weighed. The extraction parameters were as follows: pressure 300 bar, temperature 40°C, and mass flow rate 2 kg h^{-1}. The separator operated in the following conditions: pressure 15 bar and temperature 25°C.

2.4. Analysis of the composition of plum kernels used for oil extraction

The kernel samples were analysed using the standard methods of AOAC (2006) to determine the moisture level and the content of ash, fibre, protein, and oil. The protein content was determined using the Kjeldahl method (AOAC 2006, No. 984.13). The test sample was mineralised using wet combustion (digestion) in sulphuric acid with a catalyst, followed by distillation with ammonia and titration. The nitrogen content was multiplied by a conversion factor (usually 6.25) to obtain the crude protein content. The next test was intended to determine the oil content using the Soxhlet extraction method (AOAC 2006, No. 920.39). The dry sample was extracted with petroleum ether in a Soxhlet apparatus for several hours. After evaporation of the solvent, the fat was weighed and expressed as a percentage of the dry sample weight. Next, the ash content was determined by combustion in a muffle furnace (AOAC 2006, No.

923.03). The test sample was burned at a high temperature (approximately 550°C) in a muffle furnace until all organic substances were completely removed. The weight of the mineral residue (ash) after combustion was expressed as a percentage of the total dry weight. The crude fibre content was determined using the Van Soest method (AOAC 2006, No. 962.09). The test sample was first digested with diluted sulphuric acid and then with sodium hydroxide. The residue after digestion was dried and weighed (crude fibre). The crude fibre content was expressed as a percentage of the sample weight.

2.5. Characterisation of the chemical properties of plum kernel oils

The chemical properties of the oils were determined according to official methods (American Oil Chemist's Society, AOCS, 1997) used to determine the acid value (Cd 3d-63 method), the peroxide value (Cd 8-53 method), the iodine value (Cd 1-25 method), the saponification value (Cd 3-25 method), the content of unsaponifiable matter (Ca 6a-40 method), the density (with a 10 mL pycnometer at 24°C), and the refractive index (Abbe refractometer AR3D, Krüss Optronic, Hamburg, Germany). The anisidine value was determined according to the PN-EN-ISO 6885:2008 standard. The extinction coefficients K232 (conjugated dienes) and K270 (conjugated trienes) were determined by measuring the absorbance of a 1% oil solution in cyclohexane at a wavelength of 232 and 270 nm, respectively. The absorbance was measured at room temperature (22°C) at a resolution of 1-nm in a 1 × 1 cm cuvette of a UV spectrophotometer (Shimadzu Co., Kyoto, Japan).

2.6. Fatty acid composition in plum kernel oils

The fatty acid profile of the analysed plum kernel oils was determined by gas chromatography (GC) with the use of a Shimadzu GC-17A gas chromatograph (Shimadzu, Japan). Lipid extraction was performed according to Folch *et al*. (1957). To analyse their composition, fatty acids were derivatised into fatty acid methyl esters (FAME), following the AOCS Ce 2-66 method (AOCS, 1997). Fatty acid methyl esters were determined qualitatively and quantitatively with the gas chromatography method using a SP-2560 capillary column (100 m × 0.25 mm i.d., × 0.2 μm) with a flame ionisation detector (FID) and an AOC-20i automatic injector.

The injection chamber temperature was 250°C while the detector temperature was set to 280°C. The sample volume added to the chromatography column was 1.0 μL, with a gas flow split of 1:50. The flow rates of air, hydrogen, and helium in the ionisation gas flow were 450, 40, and 30 mL min^{-1}, respectively. Helium was used as a carrier gas with a flow rate adjusted to 2.0 mL min^{-1}.

The peaks of the analysed compounds were identified by comparing their retention times with the retention times of a mixture of external standards of fatty acid methyl esters (Sigma-Aldrich, Germany). The concentration of individual fatty acids is calculated based on the comparison of their peak areas to the peak area of the internal standard. The results are usually expressed as a percentage of the total fatty acid content or as an absolute concentration (*e.g*. mg g^{-1} of sample), depending on the purpose of the analysis. Heptadecanoic acid (C17:0) was used as an internal standard. The fatty acid analysis was performed in triplicate for each sample, and the results were expressed as mean values in a percentage.

2.7. Determination of tocopherol content

The analysis of the composition of tocopherols (α, γ, and δ) was performed by normal-phase high performance liquid chromatography (NP-HPLC, UV-Vis Detectors, SPD-40) according to the methodology described in Food Analytical Chemistry Methods (Wrolstad, 2003). 0.1 g of extracted plum kernel oils and 0.05 g of ascorbic acid were added to a test tube; 5 mL of 90% ethanol and 0.5 ml of an 80% aqueous KOH solution were than added. The mixture was vortexed for 30 s, and the test tube was sealed and incubated in a water bath (70°C) for 30 min, including the time of periodic vortexing. The samples were then cooled in an ice bath (CB-200, Cole-Parmer, Illinois, USA) for approx. 5 min. In the next step, 3 mL of deionised water and 5 mL of n-hexane were added to the tube and the mixture was vortexed for 30 s. The sample was then centrifuged at 4000 rpm for 10 min at room temperature and the upper hexane layer was transferred to another tube. The other part, *i.e.* the aqueous layer with the residue, was subjected to a new extraction process according to procedure described above. The upper hexane layers from both extractions were pooled and the solvent was evaporated by purging with nitrogen. The extracted material was redissolved in 1 mL of hexane/ethyl acetate/acetic acid (mobile phase), vortexed for 30 s, and transferred to an HPLC separation vessel (Nexera Lite, Shimadzu, Japan). The size of the sample to be separated was 20 μL. The separation was performed on a Supelco Sil LC-Si column (250 x 4.6 mm, Supelco Inc.). The mobile phase consisting of hexane/ethyl acetate/acetic acid (98:1:1 v/v/v) was used at a flow rate of 1.5 mL min^{-1}. The tocopherols were determined at a wavelength of 295 nm. Qualitative analysis was performed by comparing their retention times with those of the pure standards (α-, γ-, and δ-tocopherols). Their quantity, in turn, was estimated using their peak areas.

2.8. Determination of phytosterol content

Phytosterols were determined using the GC technique according to the methodology described by Rudzińska *et al*. (2001). Using this method, phytosterols were extracted from 1 g of ground seeds with Folch's solution (a solution of chloroform and methanol 2:1, v/v). Next, 500 μg of 5-α-cholestane was added to the extracted fat as an internal

standard. The sample was then saponified with methanolic 1 M KOH at room temperature for 18 h. The unsaponified fraction was extracted with diethyl ether. The plant sterols were analysed as TMS derivatives using a GC-2050 BREVIS gas chromatograph and a DB5 column (30 m × 0.32 mm × 0.25 μm). The injector and detector temperatures were 310 ± 1°C and samples were injected in the split mode (1:25). The analyses were performed at a constant temperature of 290 ± 1°C with a constant helium flow of 1.6 mL min^{-1}. The phytosterols were identified on the basis of their retention times compared to the corresponding standards.

2.9. Determination of amygdalin content

Two grams of plum kernel oils were weighed and transferred to a round-bottom flask containing 50 mL ethanol. The mixture was heated under reflux for 120 min. The extracts were then filtered, and the ethanol was completely evaporated under vacuum. The samples were mixed with 10 mL diethyl ether and vortexed for 1 min to precipitate amygdalin. Diethyl ether was evaporated on a rotary evaporator (Hei-VAP Expert Model, Heidolph, Germany). The residue was dissolved in 5 mL of distilled water. Immediately before HPLC analysis, the samples were filtered through a 0.2 μm PTFE filter. The separation was performed using the Nexera Lite HPLC system (Shimadzu, Japan). The size of the analysed sample was 20 μL. The detector UV was set to a wavelength of 210 nm. A C18 column (4.6 mm × 250 mm, 5 μm) kept at 20°C was used for the separation procedure. Quantitative analysis was performed by measuring the peak areas. RP-HPLC analysis was performed using isocratic elution with a flow rate of 1.0 mL min^{-1}. The mobile phase was water: methanol (75:25 v/v). The separation was performed in triplicate.

2.10. Total polyphenol content and antioxidant properties

2.10.1. Total polyphenol content

The total polyphenol content was determined with the Folin-Ciocalteu reagent according to the method described by Gutfinger (1981) with minor modifications. The methanol extract of the oil samples (20 μL) was mixed with 2.5 mL Folin-Ciocalteu reagent (diluted 1x10). After 3 min, 2 mL of 7.5% anhydrous sodium carbonate was added. In the next step, the samples were incubated for 2 h in the dark at room temperature. After this time, the absorbance of the samples was measured at 765 nm using a UV-2600i plus spectrophotometer (Shimadzu, Japan). The results were expressed as mg gallic acid GAE 100 mL^{-1} oil.

2.10.2. Antioxidant properties: DPPH and ABTS assays

DPPH assay: The DPPH radical scavenging capacity (2,2-diphenyl-1-picrylhydrazyl) was determined as in Bondet *et al*. (1997) with minor modifications. The oil samples (20 mg) were placed in a test tube and mixed with 80 μL ethyl acetate and 2.9 mL of a DPPH solution. The samples were mixed for 20 s in a vortex mixer (Fisherbrand™ ZX3, Fisher Scientific, USA). After incubation in the dark for 30 min, the absorbance of the samples was measured at a wavelength of 520 nm. The measurement was made in relation to ethyl acetate. The results were expressed in mg Trolox 100 mL^{-1} oil.

ABTS assay: The ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-) sulfonic acid) radical scavenging capacity was determined according to the method described by Re *et al*. (1999) with minor modifications. The $ABTS^{+}$ stock solution was prepared by reacting a 7.0 mM ABTS stock solution with 2.45 mM potassium persulfate in the dark for 16 h. The solution was diluted with ethanol and the absorbance was adjusted to 0.700 ± 0.020 at 765 nm. A 100-μL oil sample was spiked with 2.9 mL of the diluted $ABTS^{+}$ solution. The resulting solution was mixed for 20 s using a vortex mixer (Fisherbrand™ ZX3, Fisher Scientific, USA). The absorbance was measured after 6 min at a wavelength of 765 nm. The results were expressed as mg Trolox 100 mL^{-1} oil.

2.11. Statistical analysis

All determinations were performed in triplicate and the values were expressed as the mean of three measurements ± standard deviation. Statistical analysis was performed using Statistica software (v.13.3, Statsoft, USA). Analysis of variance (one-way ANOVA) was performed to test for differences in the means, followed by Tukey's HSD test at a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

The chemical composition of plum (*Prunus domestica* L.) kernels is important, as it determines the nutritional value and quality of the final product. Unconventional oily kernels can be a very good source of oil rich in tocopherols, phytosterols, antioxidants, and other bioactive components (Ćirković *et al.,* 2023). The moisture and content of oil, protein, ash, and fibre in the plum kernels studied are shown in Table 1. The average moisture content in the kernels of 'Diana' plums was 4.02%. The average oil content of the plum kernels was 30.01%. The present results were similar to those reported by Anwar *et al*. (2014). A significantly higher oil level (46.3-55.5%) was found in plum kernels

Table 1. Proximate analysis of plum (*Prunus domestica* L.) kernels

Constituent	Content (%)
Oil (kernel)	30.01±0.064
Moisture	4.02±0.026
Protein	13.86±0.04
Ash	2.22±0.03
Fiber	6.01±0.045

Result ± standard deviation.

by Matthäus and Özcan (2009). These discrepancies may be related to differences in agroclimatic conditions or plum varieties. The analysis of the oilseed residues after the oil extraction revealed an average protein content of 13.86% in the plum kernels. The high protein content in the plum kernels indicates that this material can be a source of plant protein that may be added to feed for livestock or poultry. Compared to the present study, Ćirković *et al*. (2023) reported significantly higher protein contents in plum kernels, namely 26.94%. The protein content in plum seeds (*Prunus domestica* L.) can vary and depends on many biological, environmental, and technological factors. The key factors influencing the protein content include the plum variety, climatic and soil conditions, fruit ripeness at harvest, storage conditions, drying methods, technological processing, biotic and abiotic stresses, and the use of fertilisers and plant protection products. The ash and fibre content in the kernels of the 'Diana' variety was 2.22 and 6.01%, respectively.

3.1. Physicochemical characteristics of plum kernel oils

The physicochemical properties of the plum kernel oils obtained using the various methods are shown in Table 2. The moisture content in oils is of great importance, as its high level can lead to accelerated oil aging and oxidation and promote the growth and development of microorganisms (Mafe *et al.,* 2024). In addition, the moisture content in oils affects their processing (Nwakodo *et al.,* 2018). The value of this parameter in the plum kernel oils extracted in this study ranged from 0.34 to 0.81%; it was lowest in the SFT (supercritical CO_2 extraction) oil and the highest in the OH (oil extraction with n-hexane**)** oil. Based on these results, it can be assumed that the SFT oil had the highest stability than the other products (Dimzon *et al.,* 2011). The density of the extracted oils (24°C) ranged from 0.84 to 0.92 g cm^{-3}, with the SFT oil having the highest level and the OH oil exhibiting the lowest value. These differences were statistically significant ($p < 0.05$). In turn, the refractive index (RI) values (40°C) of the plum kernel oils were 1.4437 (OH), 1.4442 (PSO), and 1.4475 (SFT). RI values within the expected range indicate high purity and adequate oil quality. Deviations from the standard range may suggest the presence of contaminants, oxidation, or other chemical changes that may affect the stability and shelf life of the oil. In industrial practice, monitoring the refractive index is a useful quality control tool that allows rapid detection of potential abnormalities in the oil composition. Regular RI measurements can help ensure production consistency and maintain high standards of quality in the final product. The RI value is influenced by such factors as the plum variety, extraction methods, and oil refining degree. The refractive index is an important physicochemical parameter that reflects the optical density and chemical composition of oil.

The iodine value in the oils was in the range from 98.04 g I_2 100 g^{-1} oil to 102.97 g I_2 100 g^{-1} oil.

Several factors influence the iodine value of oils, including the fatty acid composition, type of plant raw material, cultivation conditions, climate, extraction and refining processes, and oil oxidation degree. The iodine value reflects the amount of halogen (in grams) that is attached to the double bonds of the fatty acids contained in 100 g of a product in specific conditions (Firestone, 1994). Similar iodine values of cold pressed plum kernel oils were reported by Anwar *et al*. (2014). Another parameter determined in the present study was the saponification value, which allows the average molecular weight of fatty acids to be determined. It is the amount of KOH, expressed in milligrams, required to saponify esterified fatty acids and neutralise free fatty acids contained in 1 g of fat. The saponification value is an important analytical parameter, especially in

Table 2. Physicochemical properties of plum (*Prunus domestica* L.) seed oils extracted with different methods

Component	Oil extraction (OH)[a]	Cold pressing (PSO)[b]	Supercritical CO_2 extraction (SFT)[c]
Moisture (%)	0.81±0.01[bc]	0.58±0.025[ac]	0.34±0.021[ab]
Refractive index (40°C)	1.4437±0.0002[c]	1.4442±0.0003[c]	1.4475±0.0005[a,b]
Density mg mL^{-1} (24°C)	0.84±0.01[b,c]	0.88±0.006[a,c]	0.92±0.006[a,b]
Saponification values (mg of KOH g^{-1} oil)	150.86±0.28[b,c]	162.16±0.39[a,c]	165.26±0.17[a,b]
Unsaponifiable matter (%)	0.87±0.011[b,c]	0.93±0.011[a]	0.94±0.06[a]
Acid value (mg KOH g^{-1} oil)			
Iodine value (g I_2 100 g^{-1} oil)	98.04±0.26[b,c]	99.44±0.09[a,c]	102.97±0.19[a,b]
Peroxide value (meq O_2 kg^{-1} oil)	1.24±0.01[b,c]	1.19±0.01[a,c]	1.15±0.006[a,b]
Para-anisidine value	1.11±0.006[c]	1.10±0.00[c]	1.03±0.012[a,b]
Conjugated Dienes (λ232)	1.741±0.005[b,c]	1.994±0.006[a,c]	2.178±0.002[a,b]
Conjugated Trienes (λ270)	0.883±0.002[b,c]	0.9986±0.001[a,c]	1.1769±0.004[a,b]

Values are mean ± SD of triplicate determinations. Different letters in superscript within the same row indicate significant differences among the variants.

raw material quality control, cosmetic formulations, and detection of possible adulterations. Potassium hydroxide accelerates fat hydrolysis (Ivanova *et al.,* 2022). The OH oil had the lowest saponification value, *i.e.* 150.86 mg of KOH g^{-1} oil, while the SFT oil had the highest value of this parameter, *i.e.* 165.26 mg KOH g^{-1} oil. These results were similar to those reported by Anwar *et al*. (2014). The highest amount of unsaponifiable matter, *i.e.* 0.94%, was found in the SFT oil. The cold pressed PSO oil had a similar value (0.93%). These values were similar to those determined by other researchers (Anwar *et al*., 2014).

The peroxide value, which is a measure of primary oxidation products, was low in the plum kernel oils, ranging from 1.15 (SFT) to 1.24 (OH) meq kg^{-1} (Zhang *et al*., 2021). The peroxide value is a vital indicator of oil quality and suitability for consumption. Routine monitoring of this parameter helps prevent the ingestion of rancid fats and ensures the preservation of the nutritional value of oils. It should be emphasised that an unpleasant odour and a rancid taste of oils are primarily caused by the presence of secondary aldehyde products, and their content in oils can be determined by estimating the para-anisidine value (Tarapoulouzi *et al.,* 2022). Factors influencing the p-AV in plum seed oil include the extraction method, thermal processing, exposure to oxygen and light, and antioxidant content. Oils with a robust antioxidant profile exhibit enhanced oxidative stability. The amount of secondary oxidation products, expressed by the para-anisidine value, was low in all the oils studied, indicating a good oxidation state. This value was 1.03 for the SFT oil, 1.10 for the PSO oil, and 1.11 for the OH oil. The characteristic absorbance at 232 and 270 nm, based on dienes and trienes, was 1.741 and 0.883 for the chemically extracted oil, 1.994 and 0.9986 for the cold-pressed oil, and 2.178 and 1.1769 for the supercritical CO_2 extraction variant, respectively.

The oil extraction method plays a key role and can significantly affect the final chemical composition of oils, including the content and proportions of fatty acids. Different extraction techniques vary in their ability to extract lipids and accompanying compounds, which has a direct impact on the quality and functional properties of oils. The extraction method is important because it affects the lipid yield – chemical methods (*e.g*., n-hexane extraction) typically provide higher oil yields compared to mechanical methods (*e.g*., cold pressing). In addition, the extraction method affects the fatty acid profile, the content of accompanying compounds (tocopherols, sterols, and phenols), and the sensory and physicochemical properties of the oil, including taste, colour, oxidative stability, and nutritional value. These results indicate that the oils are characterised by high resistance to secondary oxidation. The spectrophotometric measurement of specific absorption (free absorption) serves as an important parameter in the evaluation of oil quality with regard to the presence of secondary oxidative changes (Rossell, 1991).

3.2. Fatty acid profile in plum kernel oils

Table 3 shows the percentage content of fatty acids in the plum kernel oils obtained with the different methods. Thirteen fatty acids were detected in the tested oils. Oleic acid and linoleic acid accounted for the highest percentage in all the oils. The highest oleic acid content, *i.e.* 67.98%,

Table 3. Fatty acid profile in plum (*Prunus domestica* L.) seed oils extracted with different methods

Number of carbons and unsaturated bonds in fatty acids	Name of fatty acid	Organic oil extraction (OH)[a]	Cold pressing (PSO)[b]	Supercritical CO_2 extraction (SFT)[c]
		(%)		
C16:0	Palmitic acid	5.84±0.01[b,c]	5.46±0.04[a,c]	5.16±0.015[a,b]
C16:1 (cis-9)	Palmitoleic acid	0.88±0.03[b,c]	0.93±0.006[a]	0.96±0.006[a]
C17:1 (cis-10)	Heptadecenoic acid	0.088±0.003[b,c]	0.11±0.006[a]	0.12±0.000[a]
C18:0	Stearic acid	2.14±0.02[b,c]	1.59±0.02[a,c]	1.15±0.02[a,b]
C18:1 (cis-9)	Oleic acid	65.94±0.03[b,c]	66.27±0.047[a,c]	67.98±0.075[a,b]
C18:2 (cis-9,12)	Linoleic acid	24.88±0.03[b,c]	25.49±0.015[a,c]	24.47±0.072[a,b]
C20:0	Arachidic acid	0.093±0.006[b,c]	0.06±0.003[a]	0.053±0.006[a]
C18:3 (cis-9,12,15)	Linolenic acid	0.053±0.006[b,c]	0.08±0.005[a,c]	0.097±0.006[a,b]
C22:1 (cis-13)	Erucic acid	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
C24:0	Lignoceric acid	0.01±0.00	nb	nb
Σ Saturated fatty acids SFA		8.083±0.028[b,c]	7.110±0.077[a,c]	6.363±0.121[a,b]
Σ Unsaturated fatty acids UFA		91.851±0.03	92.89±0.05	93.637±0.09
Σ Monounsaturated fatty acids MUFA		66.918±0.03[c]	67.32±0.05	69.07±0.08
Σ Polyunsaturated fatty acids PUFA		24.933±0.005	25.570±0.004	24.567±0.006

Explanations as in Table 2.

Table 4. Percentage content of sterols in plum (*Prunus domestica* L.) seed oils extracted with different methods

Sterols	Organic oil extraction (OH)[a]	Cold pressing (PSO)[b]	Supercritical CO_2 extraction (SFT)[c]
Campesterol	5.55±0.015[b,c]	5.88±0.03[a,c]	5.76±0.03[a,b]
Stigmasterol	1.34±0.021[b,c]	1.89±0.02[a]	1.90±0.02[a]
β-Sitosterol	84.14±0.083[b,c]	82.61±0.06[a,c]	83.28±0.07[a,b]
D7-Stigmastenol	0.10±0.006[a]	0.1±0.00[a]	0.11±0.01[a]
Δ5-Avenasterol	7.90±0.031[b]	8.18±0.05[a,c]	7.96±0.05[b]
Δ7-Avenasterol	0.96±0.021[a]	0.95±0.006[a]	0.99±0.017[a]

Explanations as in Table 2.

Table 5. Tocopherol content (mg/kg) in plum (*Prunus domestica* L.) seed oils extracted with different methods

Tocopherols	Organic oil extraction (OH)[a]	Cold pressing (PSO)[b]	Supercritical CO_2 extraction (SFT)[c]
α-tocopherol	47.80±0.04[b,c]	48.62±0.10[a,c]	55.22±0.10[a,b]
γ-tocopherol	186.05±2.93[c]	191.60±1.41[c]	222.65±2.51 [a,b]
δ-tocopherol	220.88±0.31[b,c]	230.03±0.32[a,c]	242.09±0.75 [a,b]
Total	454.73±3.19[b,c]	470.25±1.62[a,c]	519.96±3.00 [a,b]

Explanations as in Table 2.

was recorded for the SFT oil. In contrast, the highest level of linolenic acid was recorded in PSO at 25.49%. Similar results were obtained by Natić *et al*. (2020), who confirmed in their research that oleic and linoleic acids were dominant in plum kernel oil. These results are also consistent with those reported by Kiralan *et al*. (2018), who found the highest amounts of oleic acid, followed by linoleic and palmitic acid in plum kernel oils obtained in their study. Similarly, Vladić *et al*. (2020) found that oleic acid and linoleic acid were the dominant fatty acids in plum kernel oil. Oleic acid contributes to lowering triglyceride and total cholesterol levels. It is noteworthy that the high oxidative stability of vegetable oil is very often attributed to high concentrations of oleic acid (Abdulkarim *et al*., 2007; Uluata, 2016). In the present study, the OH oil had the highest concentration of saturated acids, *i.e.* palmitic acid (5.84%), stearic acid (2.14%), and arachidonic acid (0.093%).

In their study, Matthäus and Özcan (2009) determined an oleic acid content of 66.9% and a linoleic acid content of 22.7% in plum kernel oil, which is similar to the present results. In view of the low content of saturated fatty acids, the high content of monounsaturated oleic acid and the desirable composition of fatty acids, plum kernel oil is recommended as edible oil suitable for human consumption (Matthäus and Özcan, 2009).

3.3. Composition of phytosterols in plum kernel oil extracted with different methods

The qualitative and quantitative composition of the phytosterols in the plum kernel oils obtained with the various methods is shown in Table 4. Six sterols were identified in the oils: campesterol, stigmasterol, β-sitosterol, D7-stigmastenol, Δ5-avenasterol, and Δ7-avenasterol. β-sitosterol was the most abundant phytosterol in all the plum kernel oils, with the range of 82.61% (PSO) – 84.14% (OH), followed by Δ5-avenasterol ranging from 7.90% (OH) to 8.18% (PSO). β-sitosterol is considered the major plant sterol with the greatest abundance in plant seed oils. A study conducted by Hassanin (1999) also confirmed that β-sitosterol was the major sterol in Egyptian plum kernel oils (87.4%). There is information in the literature on the health-promoting properties of phytosterols, which contribute in particular to reducing the incidence of prostate cancer and elevated cholesterol levels as well as modulating and improving the immune function of the organism (Anwar *et al*., 2008; Rossell, 1991).

3.4. Tocopherol content

The tocopherol content in the plum kernel oils extracted with the different methods is shown in Table 5. Three tocopherol isomers (α-, γ-, and δ-) were identified. The highest content of α-tocopherol, δ-tocopherol, and γ-tocopherol was determined in the SFT oil, *i.e*. 55.22, 222.65, and 242.09 mg kg^{-1}, respectively. In contrast, the lowest tocopherol contents were found in the oil extracted using the n-hexane extraction method. Similar results were reported by Vladić *et al*. (2020). It was found that the content of tocopherols in the oil analysed in this study was higher than that in seed oils extracted from plums grown in Egypt (Hassanin *et al.,* 1999). It is noteworthy that δ-tocopherol is a more effective antioxidant than α- and γ-tocopherol, while α-tocopherol has a higher vitamin E effect (Anwar *et al.,* 2008). The substantially higher concentration of tocopherols in the extracted plum kernel oils may contribute

Table 6. Polyphenol content and antioxidant activity (DPPH and ABTS assays) of plum (*Prunus domestica* L.) seed oils extracted with different methods

	Organic oil extraction (OH)[a]	Cold pressing (PSO)[b]	Supercritical CO_2 extraction (SFT)[c]
Total polyphenols (mg GAE 100 mL^{-1})	3.03±0.04[b,c]	3.54±0.02[a,c]	3.64±0.02[a,b]
DPPH (mg Trolox 100 mL^{-1})	22.79±0.15[b,c]	24.45±0.12[a,c]	26.48±0.15[a,b]
ABTS (mg Trolox 100 mL^{-1})	39.28±0.06[b,c]	42.39±0.04[a,c]	44.21±0.04[a,b]

Explanations as in Table 2.

to their good oxidative stability and nutritional quality. A significantly lower concentration of α-tocopherol (2.83 mg 100 g^{-1}) was reported by Ćirković *et al*. (2023) in their studies on cold-pressed plum kernel oil. The α-tocopherol content in plum kernel oil is influenced by a range of both biological and technological factors, including the plum cultivar, climatic and soil conditions, fruit ripeness, kernel storage conditions, the oil itself, contamination, and raw material quality.

3.5. Total polyphenol content and antioxidant properties of plum kernel oils

The formation of free radicals in vegetable oils, which leads to a deterioration in their quality, cannot be completely avoided. However, the content of phenolic compounds has an influence on the quality, stability, and nutritional value of the oils (Sigeri *et al.,* 2008).

The total polyphenol content and antioxidant properties of the obtained oils are shown in Table 6. The total polyphenol content in the tested oils ranged from 3.03 to 3.64 mg GAE 100 mL^{-1}, with the highest amount found in the SFT oil and the lowest content in the chemically extracted oil (OH). Similar results were obtained by Ćirković *et al*. (2023) in their study on cold-pressed plum kernel oil.

The determination of the antioxidant capacity of oils is highly important, as the value of this parameter provides information about the content of bioactive compounds, *e.g.* unsaturated fatty acids, tocopherols, and others (Bjelica *et al*., 2019). Two assays: DPPH and ABTS were used to determine the antioxidant properties of the extracted oils. Both assays showed the highest antioxidant activity of the SFT oil. In the DPPH method, the antioxidant activity of the oils ranged from 22.79 to 26.48 mg Trolox 100 mL^{-1}. The highest antioxidant activity values, as determined by the DPPH and ABTS assays, were observed for the plum kernel oil extracted using supercritical carbon dioxide (CO_2) due to the high selectivity and mild conditions of this method. Supercritical CO_2 extraction enables efficient recovery of bioactive compounds, such as tocopherols, carotenoids, and phenolic substances, while minimising their thermal and oxidative degradation. As a result, the obtained oil exhibits a higher concentration of natural antioxidants, which translates into enhanced free radical scavenging capacity in both assays. Additionally, the absence of organic solvents during extraction eliminates the risk of product contamination, further contributing to the preservation of its antioxidant potential. In turn, the antioxidant activity determined with the ABTS method ranged from 39.28 to 44.21 mg Trolox 100 mL^{-1}. Similar values of antioxidant activity, *i.e.* 63.3 mg/Trolox 100 g oil, were reported by Uluata and Özdemir (2017) in a study on cold-pressed oil from plum seeds from Turkey. Fratianni *et al*. (2018) analysed plum kernel oil and reported its 71% antioxidant activity in the DPPH assay.

3.6. Amygdalin content

Amygdalin, a cyanide glycoside that occurs naturally in bitter almonds and the seeds of many fruits, is a controversial substance. Although it has proven toxic properties, it is used in alternative medicine as an adjuvant substance in cancer treatment (Vladić *et al*., 2020). Unhydrolysed amygdalin has no toxic effect on the organism, but its degradation products, especially hydrocyanic acid, are toxic. It has also been shown that the toxicity of amygdalin associated with the release of hydrocyanic acid requires the microbiological activity of the intestinal flora. Humans and herbivores produce enzymes such as rhodanase and hydroxocobalamin, which are able to detoxify hydrocyanic acid and transform it into much less toxic compounds (He *et al*., 2016). The content of amygdalin in the seeds of the 'Diana' variety and in the oils extracted from this raw material using the different methods is shown in Table 7. The average content of amygdalin in the kernels was 0.321 mg g^{-1}.

Table 7. Amygdalin content in plum (*Prunus domestica* L.) kernels and plum seed oils extracted with different methods

Sample	Amygdalin content
Plum kernels	0.32100±0.0032 (mg g^{-1} kernels)
Organic extraction (OH)	0.00810±0.0004 (mg g^{-1} oil)
Cold pressing (PSO)	0.00480±0.0003 (mg g^{-1} oil)
Supercritical CO_2 Extraction (SFT)	0.00082±0.000 (mg g^{-1} oil)

Limit of detection – 0.00025 mg g^{-1}, Limit of quantitation – 0.00075 mg g^{-1}. Other explanations as in Table 2.

Similar results were reported by Vladić *et al*. (2020) in their study on plum kernels. A higher amygdalin content in plum kernels (4.39 mg g^{-1}) was reported by García *et al*. (2016). These significant differences can be attributed to environmental factors, *e.g*. the geographical location, weather conditions, and analytical methods used (Senica *et al.,* 2017). The amygdalin content was significantly lower in all the tested oils than in the fresh kernels. During oil extraction, especially using the cold pressing method, it is possible for small amounts of certain compounds to be transferred from the plant material to the oil. This also applies to amygdalin, a natural cyanogenic glycoside found in apricot kernels, bitter almonds, apple seeds, and plums, among other things. Amygdalin is soluble in water but not in fats, which means that its solubility in oil is limited. In practice, this means that, during cold pressing, where no high temperatures or chemical solvents are used, only trace amounts of amygdalin are transferred – usually at levels that are harmless to health. The amygdalin content in oil depends on the type of raw material and the degree of its purification. For example, when pressing oil from whole plum kernels, the risk of amygdalin presence is greater than when pressing from previously separated pulp. The amygdalin content in oil is influenced by the type of raw material, the degree of processing of the raw material, and the extraction method. The SFT oil had a very low amygdalin content of only 0.00082 mg g^{-1} oil. The amygdalin content in the oils obtained by extraction with n-hexane and cold pressing was 0.0081 and 0.0048 mg g^{-1} oil, respectively. The reduction in the amygdalin content in the oils compared to that in the kernels can be explained by the enzymatic degradation of amygdalin to hydrocyanic acid. Since the SFT and PSO oil extraction was carried out at a low temperature (40ºC), the enzymes were not inactivated, which may have contributed to the low amygdalin content (Bolarinwa *et al.,* 2014).

4. CONCLUSIONS

Despite the increasing number of studies on the extraction of non-conventional oils from fruit kernels, there is still a problem with waste disposal. Solving this problem will be an essential basis for improving the productivity and sustainability of the food production system. The high oil content in plum kernels is comparable to that of commercial oilseeds, such as rapeseed or sunflower. The average oil content of the investigated plum kernels was 30.01%. The following parameters were determined in the extracted plum kernel oils: iodine value: 98.04-102.97 g I_2 100 g^{-1} oil, density (at 24°C) 0.84-0.92 mg mL^{-1}, refractive index (40°C) 1.4437-1.4475, saponification value 150.86-165.26 mg KOH g^{-1} oil, and unsaponifiable matter 0.87-0.94%. The specific absorbance values at 232 and 270 nm were 1.741-2.178 and 0.883-1.1769, respectively, while the peroxide value was 1.19-1.24 meq O_2 kg^{-1}. Oleic acid and linoleic acid were the major fatty acids detected in all the tested oils. The total tocopherol content in the plum kernel oil ranged from 454.73 mg kg^{-1} (OH) to 519.96 mg kg^{-1} (SFT). The dominant tocopherol in the oils was δ-tocopherol, which was most abundant in the SFT oil. β-sitosterol and Δ5-avenasterol were the major phytosterols in the oils. The SFT oil had the highest content of total phenols and the strongest antioxidant properties in both the DPPH and ABTS assays. Therefore, plum kernels are a promising by-product for oil extraction. The physicochemical composition, lipid profile, tocopherol and phytosterol content, and antioxidant properties of the oil obtained with the supercritical CO_2 extraction are significantly more beneficial than these parameters of the oils obtained by chemical extraction and cold pressing. In addition, the oil obtained using the supercritical CO_2 extraction technique was characterised by a higher yield and a very low amygdalin content. It can therefore be used in both nutritional and pharmaceutical applications.

Conflict of interest. The authors declare that they have no conflict of interest.

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