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Research Article

Processing waste: bioactive components and antioxidant capacity of cold-pressed oils from some fruit seeds

Aleksandra ĆIRKOVIĆ, Mirjana DEMIN, Milica FOTIRIĆ AKŠIĆ, Biljana RABRENOVIĆ*

*University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia; cirkovicco@gmail.com; demin@agrif.bg.ac.rs; fotiric@agrif.bg.ac.rs; biljanar@agrif.bg.ac.rs (*corresponding author)*

Abstract

In this study, the possible use of seeds after fruit processing to obtain unconventional cold-pressed edible oil was investigated. For this purpose, seeds of quince, sour cherries and plum were used. Fatty acid composition, antioxidant activity, peroxide and acid value, oxidative stability, and tocopherols and phytosterols content were determined in the studied oils. Plum seed oil was dominated by oleic acid, while quince and sour cherry seed oils contained abundant linoleic acid. The total polyphenol content in the studied oils ranged from 2.28 to 9.03 mg GAE 100 mL^{-1}. Antioxidant properties (ABTS, DPPH, FRP) were associated with tocopherol content. All three studied oils were rich in β-sitosterol. The oxidative stability of the studied oil samples varied and ranged from 1.92 h-31.17 h. Quince seed oil had the highest content of α-tocopherol (44.30 mg 100 g^{-1}) and plum seed oil had the lowest (3 mg 100 g^{-1}), while sour cherry seed oil had the highest content of β+γ-tocopherol (17.19 mg 100 g^{-1}). The results show that oil from quince, sour cherry, and plum seeds is suitable for the production of a high quality cold-pressed oil. The use of this type of waste from fruit processing contributes to waste reduction and promotes the circular economy.

Keywords: bioactive components; circular economy; cold-pressed oil; plum; seeds; sour cherry; quince

Introduction

Food waste (FW) is one of the biggest global problems. It can occur at any stage of the food production chain. FW is organic material that is recycled or used in other ways only to a limited extent. The quantity and quality depend on the raw materials used, the technology, and the final product's nature. Due to their organic nature and aerobic reaction products, they pose a health risk to living organisms and have harmful effects on the environment (Anal, 2017).

Over time, this problem has been mitigated mainly by converting them into compost to produce biofertilizers. Today, there are many other approaches that are classified as green technology (Figure 1). These include landfilling and recycling as a soil amendment, such as biochar, as a source of cellulose, hemicellulose, lignin, polysaccharides, and others (Banerjee *et al.*, 2017). However, it could also be a valuable resource for

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bioactive constituents. Due to the heterogeneous nature and composition of FW, the main constituents, such as lipids, proteins, and carbohydrates, were frequently found.

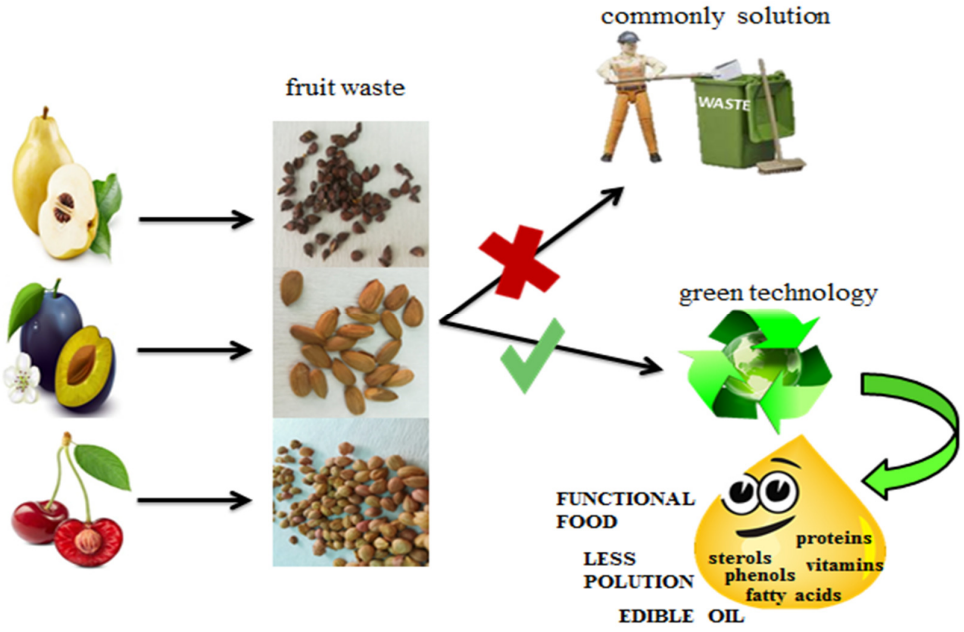

Figure 1. Potential uses of fruit waste: green technology versus commonly solutions

Today's lifestyle requires increased environmental protection awareness and increased demand for functional products in all industries (Esparza *et al*., 2020). The main fruit-processing products are jams, juices, alcoholic beverages, dried fruits, canned fruits, etc. After this type of production, a considerable amount of waste remains. This waste includes seeds, pomace, steams, pulp, cores, and leaves. The most commonly recycled FW is pomace containing seeds, cores, peels, skins, and leaves of apples, bananas, citrus fruits, grapes, and melons (Campos *et al*., 2020), which accounts for about 16% of total food waste. FW is traditionally composted or used as livestock feed. An important by-product of industrial apple processing is the pomace, which contains 95% peel/flesh, 4% seeds, and 1% stems. The pomace contains about 60% dietary fiber. This waste contains phenolic components, vitamins, carbohydrates, proteins, alkaloids, fatty acids, and sterols (Coman *et al*., 2020). Quince processing wastes are pomace, seeds, leaves and mucilage, the amount of which depends on the processing method. Dried pomace has already been used as a source for pectin extraction. Discarding seeds when processing quince is common, although they contain abundant bioactive compounds. Quince mucilage has long been used in medicine (skin wounds) and cosmetics (Urbanavičiūtė *et al*., 2019). The second use is to obtain cold-pressed oil from the seeds, which are a waste product. As a waste product, the seeds account for about 9% of the fresh fruit. Despite the low yield, quince seeds contain 6-17% oil and are rich in essential fatty acids, tocopherols, and sterols (Mišina *et al*., 2020). The waste products of plum processing are pits and pomace. The seeds of plum pits have a high content of proteins and oil, so they can be used in the food and pharmaceutical industries (González-García *et al*., 2014). During the processing of sour cherries, a considerable amount of waste is produced, namely the pits. The pits contain seeds which are rich in bioactive components, including carbohydrates (46%), proteins (29%) and lipids (17%) (Yılmaz and Gökmen, 2013). The numerous benefits to human health have been confirmed in numerous research studies (Bak *et al.,* 2006; Bak *et al.,* 2011). No toxic effects have been observed in animals or humans after the consumption of sour cherry pits (Kasapoğlu *et al*., 2021). The pits comprise 7-10% of the fresh sour cherry fruit. There are several uses for this type of waste. One is the extraction of oil from the seeds of the pits; others are their use as fertilizers, as adsorbents for Cr(VI)

in polluted waters due to their high porosity and carbon content (Yılmaz *et al*., 2018). Seeds are extremely rich in proteins and lipids consisting of fatty acids, waxes, sterols, carotenoids and vitamins (Banerjee *et al*., 2017). Edible oils are important sources of bioactive compounds with beneficial effects on human health. Cold-pressed oils have higher nutrient content than refined oils and represent a "green technology" for extraction. Researchers are focusing on finding unconventional seeds as new sources of edible oils because they are high in bioactive compounds, offer health benefits, are economically available, and can be extracted using environmentally friendly processes. Quince seeds have a high potential as a source of oil, since contain about 6.1-16.8% oil, of which about 89% are unsaturated fatty acids (Górnaś *et al*., 2014). Sour cherry seed oil is a valuable source of essential fatty acids, phytosterols and tocopherols and can be used in the food and pharmaceutical industries. However, the literature on minor bioactive compounds in this type of oil is very limited and insufficient (Górnaś *et al*., 2016).

The aim of this study was to obtain cold-pressed oil from the seed waste of three fruit species, quince, cherry and plum, and to determine the content of individual bioactive components, oxidative stability and antioxidant potential of the studied oil samples.

Materials and Methods

Material

All samples were obtained from individual fruit processors. Quince seeds were removed from core and collected as waste from juice production. Plum pits were collected from industrial drying facilities and as waste from plum processing, while sour cherry pits were collected as waste from frozen fruit production. The plum and sour cherry pits were cracked and the seeds were manually removed. All types of seeds were dried at room temperature for 24 hours. Quince, plum and sour cherry seeds were stored in plastic bags in the refrigerator until analysis.

Cold-pressing

Seeds were cold pressed in a laboratory using a screw press (OP650W, Gorenje, Slovenija). During the pressing process, the highest temperature of the outlet oil was 47 °C, which is in the line with recommendation to perform cold pressing at a temperature below 50 °C in order to preserve the bioactive components (Natić *et al.,* 2020). The oils were filtered after 24 hours, filled into 2 mL plastic Eppendorf tubes and stored in the refrigerator for further analysis. All analyses were performed in the laboratories of the Faculty of Agriculture of the University of Belgrade.

Proximate composition of the seeds

Seed samples were analysed according to AOAC (2006) standard methods for determination of moisture, ash, protein, and oil content.

Determination of Peroxide and Acid Value

Peroxide value (PV) and acid value (AV) were determined according to standard methods SRPS EN ISO 660:2015 and SRPS EN ISO 3960:2016, respectively.

Fatty acids composition

The fatty acid composition of the oils was determined according to the standard method (SRPS EN ISO12966-2: 2017). The methyl esters were separated and detected according to the standard method (SRPS EN ISO 12966-1: 2015). A gas chromatograph (Agilent Technologies 6890, USA) equipped with a split-splitless injector, a flame ionization detector (FID) and a Supelco SP-2560 capillary column (100 m length x

0.25 mm inner diameter x 0.20 μm film thickness, Supelco, Bellefonte, USA) with helium as the mobile phase and a flow rate of 5 mL/min was used. The injector and detector temperatures were 250 °C and 260 °C, respectively. The injection volume was 1 μL, and the distribution ratio of the injector was set to 20:1. The column temperature was programmed from an initial 50 °C (held for 5 min) to 240 °C (held for 20 min), with a linear temperature change of 4 °C/min. Chromatographic peaks in the sample were identified by comparing the relative retention times of fatty acid methyl esters from the samples with a standard Supelco 37-component methyl ester mixture (Supelco, Bellefonte, USA).

Indices of atherogenicity (IA) and thrombogenicity (IT) were calculated manually according to formulas (Ulbricht and Southgate, 1991):

$$IA = \frac{C12{:}\,0 + 4 \times C14{:}\,0 + C16{:}\,0}{\Sigma MUFA + \Sigma PUFA\ n-6 + \Sigma PUFA\ n-3}$$

$$IT = \frac{C14{:}\,0 + C16{:}\,0 + C18{:}\,0}{0.5\Sigma MUFA + 0.5\Sigma PUFA\ n-6 + 3\Sigma PUFA\ n-3}$$

Tocopherol content

Tocopherols were determined by HPLC (Waters M600E, USA) on a reversed-phase Nucleosil 50-5 C18 column (Machery-Nagel, Germany) with fluorescence detection using a method based on the procedure of Carpenter (1979) with some modifications. For saponification, 20 mL of 96% v/v ethanol, 0.12 g of pyrogallol, and 3 mL of KOH solution were added to 0.5 mL of oil, after which the solution was heated to 60 °C for 30 min with reflux and stirring. The contents were then cooled, transferred to a volumetric flask (50 mL) and made up to volume with ethanol.

Then an aliquot of 5 mL was transferred to a separatory funnel, and 5 mL of cold deionized water and, 5 mL of hexane were added. The mixture was shaken for 3 min, and 4 mL of the solution was dried under a nitrogen. The dry substance was then dissolved in 4 mL of methanol. The sample was filtered with a membrane syringe filter and injected into the HPLC system. The mobile phase consisted of 95% v/v methanol at a flow rate of 1.2 mL min^{-1}. Detection was performed using a fluorescence detector (Shimadzu RF -535, Japan) with an excitation wavelength of λ=290 nm and an emission wavelength of λ=330 nm. The relative retention time and maximum absorbance values at each relative retention time were used to identify tocopherols in the oil samples.

Commercial tocopherol standards were diluted appropriately and used for method validation (solution series from 0.001 to 0.5 μg mL^{-1}) and quantification (solution series from 0.05 to 10.0 μg mL^{-1}). The total time of chromatographic analysis was 20 min. The signal was processed using the "Clarify" software.

Oxidative stability

The oxidative stability of the analyzed oil samples was tested using the Oxitest instrument (Velp, Italy) according to method described by Comandini *et al.* (2009). 10 g oil samples were measured in two thermostatic titanium chambers of the instrument. The chambers are hermetically sealed. The temperature was set at 110 °C, and the initial O_2 pressure was set at 6 MPa. Special software (OXISoft, Velp, Italy) is used to monitor the pressure change in the chambers and, thus, indirectly measure the amount of oxygen consumed by the activity of each component. For each chamber, the software calculates the induction period (IP) in min or hours at the end of the test. The longer the IP, the better the oxidative stability of the sample.

Phytosterol content

The determination of phytosterols was done according to the methodology described by Verleyen (2002). In that purpose the masses of the oils 964.7 mg (QSO), 954.6 mg (PSO) and, 839.2 mg (SCSO) were measured. 500 μL of the cholesterol standard dissolved in methylene chloride was added so that the concentration of the solution was 10 mg mL^{-1}. Then, 10 mL of 50% potassium hydroxide, 28.5 mL of ethanol,

and 1.5 mL of diethyl ether were added. The mixture was refluxed at a temperature of 90 °C for 1 hour. After reflux, a liquid-liquid extraction was performed using 45 mL of hexane and 45 mL of diethyl ether. The hexane and diethyl ether fractions were combined and washed twice with 20 mL each of 0.5 M potassium hydroxide and twice with 20 mL each of 5% sodium hydroxide. The mixture was then dried with anhydrous sodium sulfate for 15 min, filtered, and the solvents evaporated.

Derivatization was maintained with 10 mg of the previously prepared mixture dissolved in 1 mL of methylene chloride. 500 µL of this solution was transferred to a vial and 50 µL of BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane) was added. Derivatization was carried out at a temperature of 60 °C for 45 min. After derivatization, the sample was analysed by gas chromatography using a mass detector.

GC and GC/MS analyses were performed using an Agilent 7890A GC equipped with a 5975C (inert XL EI /CI) MSD and a FID detector connected to the make-up via a two-way splitter using capillary flow technology. A HP -5MS capillary column (30mx0.25mmx0.25mm) was used. The temperature of the GC oven was programmed from 60 to 300 °C at 3°C/min and maintained for 10 min. Helium was used as the carrier gas at 1.5 mL/min at 60 °C (constant pressure mode). The sample was analysed in splitless mode. The injection volume was 1 mL. The temperature of the GC detector was 300 °C. MS Data were acquired in EI mode with a scan range of 30-550 m/z; the source temperature was 230 °C and the quadruple temperature was 150 °C. The solvent delay was 3 min. Identification was performed using the retention time lock (RTL) method and the RTL Adams database.

Determination of antioxidative activity

Total polyphenol content (TPC) was determined according to the procedure of Kostić *et al.* (2021) and Stikić *et al.* (2020). Briefly, 140 µL of the diluted samples were mixed with 600 µL of Folin-Ciocalteu reagent. After 5 min, 460 µL of 7.5% Na_2CO_3 was added, and this mixture was stored in a dark place for 1 h and 30 min. Before reading the absorbance, the oil samples were centrifuged at 17000 g for 5 min, and the bottom aqueous layer was separated for reading. The absorbance was measured at 765 nm. The content of total polyphenols in the samples was expressed as mg of gallic acid equivalent to 100 g of the sample FW.

The ferric reducing power (FRP), ABTS and, DPPH radical scavenging activity were determined by the same procedure (Kostić *et al.*, 2021; Stikić *et al.*, 2020). 250 µL of the samples were mixed with 250 µL of potassium phosphate buffer pH 6.6 and 250 µL of 1% potassium ferricyanide. The mixture was tempered at 50°C for 20 min. Then 250 µL of 10% trichloroacetic acid (TCA) was added to the mixture. The mixture was centrifuged, and 500 µL of the supernatant was mixed with 500 µL of water and 100 µL of 0.1% $FeCl_3$. The absorbance was measured after 10 min at 700 nm. The FRP of the samples was expressed as mg ascorbic acid equivalent to 100 g of the sample FW. In order to determine the ABTS radical scavenging activity, 100 µL of the sample was mixed with 1 mL of the ABTS working solution, which was stored in a dark place for 7 min. Absorbance was measured at 734 nm after centrifuging the sample and removing the bottom layer for reading. The ABTS activity of the samples was expressed as mg Trolox equivalent to 100 g of the sample FW. For the determination of DPPH radical scavenging activity, 105 µL of the sample was mixed with 840 µL of the previously prepared DPPH working solution. The mixture was allowed to stand in dark place for 30 min before centrifugation and removal of the bottom layer for reading. The absorbance was measured at 517nm. DPPH activity of the samples was expressed as mg Trolox equivalent to 100 g of the sample FW.

Statistical analysis

Statistical analysis was performed using statistical software STATISTICA 12. Results were expressed as mean ±SD. One-way analysis of variance (ANOVA) was performed to compare the means of the data obtained. Tukey's honestly significant difference (HSD) test was applied to determine the statistical difference between samples at a 95% confidence level ($p < 0.05$).

Results and Discussion

Proximate composition of seeds

The proximate composition of oilseeds is important because of the nutritional value, quality of the final product, potential health benefits and use in the pharmaceutical and cosmetic industries (Kurt and Atalar 2018). Unconventional oilseeds could be a good source of oil and tocopherols, phytosterols, antioxidants and other bioactive components (Sheikh *et al.*, 2022).

The tested seeds showed low moisture, ash, and high protein content, as well as a high yield of oil, which indicates appropriate quality of seeds for cold pressing. The results are expressed on a dry matter basis (Figure 2).

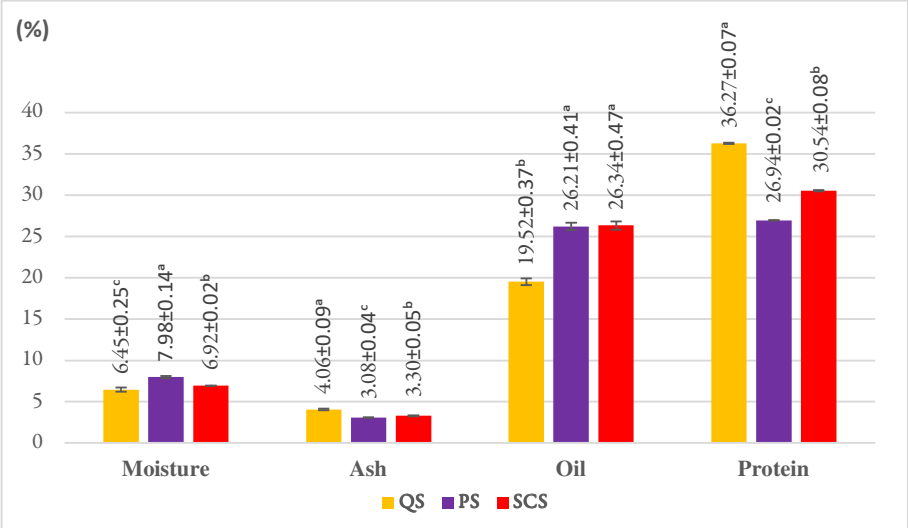

Figure 2. Composition of studied seeds

Results were presented as average ± SD (n=3). Different letters in the column indicate a significant difference according to Tukey's test ($p<0.05$), QS-quince seed; PS-plum seed; SCS-sour cherry seed.

The moisture content of the samples studied was 6.45 (QS), 6.92 (SCS) and 7.98 (PS) and was optimal for the cold-pressing process (Khan and Hanna, 1983). Based on Soxhlet extraction, PS and SCS showed a considerable oil yield of about 26%, while the oil content in QS was 19.52%. Although the oil content obtained from PS and SCS was lower than the yield reported by Uluata and Ozdemir (2017), these two seeds can be considered as economically and technologically sustainable sources of oil. The differences were probably due to the different types of PS and SCS in this work and their experiment, as well as the different growing conditions in Serbia and in Turkey. The seed samples were rich in proteins with a content of 26.94 (PS)-36.27% (QS). Ash content was in the range of 3.08 (PS)-4.06% (QS). The values of these parameters for QS were very similar to results of Kurt and Atalar (2018) who reported 35.55%, 23.56%, and 3.63% for protein, oil and ash, respectively. The parameters of PS were within the range reported by Sheikh *et al.* (2022), which were 27.57-35.91%, 36.52-50%, and 2.2-5.18% for protein, oil, and ash content, respectively. The results of this work are consistent with those of Yilmaz *et al.* (2018), who showed that SCS contained 29.3% protein, 17-36% oil, and 4.4% ash.

Peroxide (PV) and Acid value (AV)

Peroxide and acid values are important indicators of oil quality. The PV indicates the level of lipid oxidation and primary oxidation products. Unrefined vegetable oils have higher PV and AV values compared

to refined oils. AV is a measure of free fatty acid content in oils and indicates the quality of the starting raw material. The results for PV and AV of the analysed oil samples are shown in Figure 3. The values determined were within the limits recommended by the national regulations (Rulebook, 2013) for this category of oil.

The AV values were 0.28±0.01, 0.67±0.001 to 1.51±0.02 for SCSO, PSO and QSO, respectively, and were well below 4 mg KOH/g, the maximum value recommended by the national regulations (Rulebook, 2013) for cold-pressed oil. AVs for QSO (0.88 mg KOH/g) (Górnaś *et al.*, 2013), PSO (0.2-3.7 mg KOH/g) (Rabrenović *et al.*, 2021) and SCSO (1.36 mg KOH/g) (Kazempour-Samak *et al.*, 2021) can be found in the literature. The reasons for the deviation of the values compared to the literature data are probably due to different storage conditions, different parameters due to cold pressing and others. The values obtained indicate that the original seeds were of good quality regardless of the previous processing of the fruits.

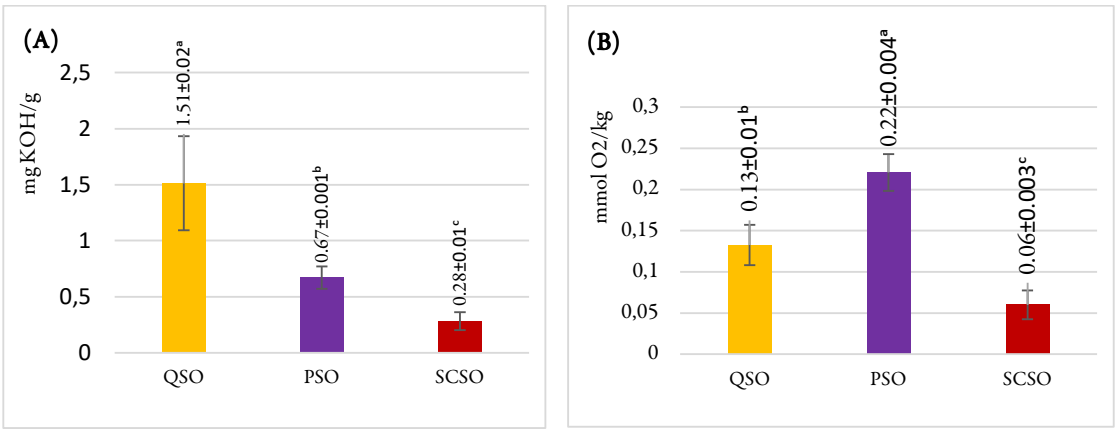

Figure 3. (**A**) AV and (**B**) PV of studied fresh oil samples with ± SD (n=3)
Error bars represent the standard deviation. Different letters indicate significant difference according to Tukey's test ($p<0.05$), QSO-quince seed oil; PSO-plum seed oil; SCSO-sour cherry seed oil.

The oil samples showed low peroxide values, 0.06±0.003 (SCSO), 0.13±0.01 (PSO), 0.22±0.004 (PSO) mmol O_2/kg, while the maximum allowable value according to the regulations is 7.5 mmol O_2/kg. Other studies resulted in PVs of 0.60 mEqO_2/kg, 0-4.3 mmolO_2/kg, and 0.99 mEqO_2/kg for QSO, PSO, and SCSO, respectively (Górnaś *et al.*, 2013; Rabrenović *et al.*, 2021; Kazempour-Samak *et al.*, 2021).

Fatty acid composition and content

Fatty acid content and composition affect the oxidative stability of cold-pressed oil. The most important factor is PUFA content, which determines the antioxidant activity of the oil (Grajzer *et al.*, 2020). In addition, inadequate intake of fatty acids can be a risk factor for numerous health problems. An increased content of saturated fatty acids can lead to an increase in cholesterol levels in the organism, while an increased content of polyunsaturated fatty acids causes low cholesterol levels. A balance between these two groups of fatty acids is a factor that can contribute to good health. The essential fatty acids, on the other hand, must be ingested with food, since they cannot be synthesized by the human organism (e.g., linoleic and linolenic acids) (Chowdhury *et al.*, 2007). The composition and content of fatty acids in the oil samples studied are shown in Table 1.

Oleic acid and linoleic acid were the major fatty acids in the samples studied. In PSO, oleic acid dominated with a content of 72.92±3.40%, while linoleic acid was represented by 20.76±2.14%. On the other hand, in QSO and SCSO, linoleic acid was the most important fatty acid with a content of 54.86±0.52% and 51.18±1.10%, while oleic acid accounted for 37.08±0.63% and 40.90±1.41%, respectively. Interestingly, saturated palmitic acid was present in all three samples with a significant percentage, ranging from 4.81±0.41% (PSO) to 5.77±1.32% (QSO). The percentage of stearic acid in saturated fatty acids was also significant, ranging from 0.74 (PSO) to 1.20% (QSO). The data obtained in this study were comparable to the results

published in the literature. Gornas *et al*. (2013) reported a linoleic fatty acid content of 52.36% in Japanese quince seed oil. When plum kernel oil was studied, Atik *et al*. (2022) and Kiralan *et al*. (2018) determined an oleic acid content of 75.4%, which is very close to the content determined in this study. The results for SCSO were similar to those reported by Yılmaz *et al*. (2020). The total SFA content was 5.77% for PSO and 7.22% for SCSO, which is in agreement with the results of Atik *et al*. (2022), who determined an SFA content of 8.40% (PSO) and 7.46% (SCSO).

Table 1. Fatty acid (FA) composition and content

FA (%)	QSO	PSO	SCSO
C14:0	0.04±0.01^{a}	0.03±0.01^{a}	0.05±0.03^{a}
C16:0	5.77±1.32^{a}	4.81±0.41^{a}	5.64±0.41^{a}
C16:1	0.03±0.0^{c}	0.37±0.06^{a}	0.14±0.04^{b}
C17:0	-	0.13±0.05	-
C17:1	0.04±0.02^{a}	0.05±0.02^{a}	0.07±0.03^{a}
C18:0	1.20±0.60^{a}	0.74±0.06^{a}	0.97±0.14^{a}
C18:1	37.08±0.63^{b}	72.92±3.40^{a}	40.90±1.41^{b}
C18:2	54.86±0.52^{a}	20.76±2.14^{c}	51.18±1.10^{b}
γ- C18:3	0.03±0.002^{a}	-	0.03±0.01^{a}
C18:3	0.17±0.03^{a}	0.03±0.02^{b}	0.10±0.03^{b}
C20:0	0.35±0.04^{b}	0.06±0.02^{c}	0.49±0.07^{a}
C20:1	0.14±0.03^{a}	0.03±0.01^{b}	0.16±0.04^{a}
C22:0	0.05±0.03^{a}	-	0.03±0.02^{a}
C24:0	0.06±0.03^{a}	-	0.04±0.02^{a}
C24:1	-	-	0.06±0.01
SFA	7.47	5.77	7.22
MUFA	37.26	73.37	41.33
PUFA	55.06	20.79	51.31
PUFA/SFA	7.37	3.60	7.11
MUFA/PUFA	0.68	3.53	0.80
AI	0.01	0.01	0.01
TI	0.03	0.02	0.02

Results are presented as average ± SD (n=3). Different letters in the column indicate a significant difference according to Tukey's test ($p<0.05$). QSO-quince seed oil; PSO-plum seed oil; SCSO-sour cherry seed oil; AI-atherogenicity index; TI-thrombogenicity index; SFA-saturated fatty acids; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids

In general, the analyzed oils were abundant in MUFA and PUFA. MUFA content was 73.37 (PSO) – 41.33% (SCSO), while PUFA content ranged from 20.79% (PSO) to 51.31% (SCSO). Our results was consistent with the data of Atik *et al*. (2022), who reported 73.72% (PSO) – 38.49% (SCSO) for MUFA, 17.88% (PSO) and 54.05% (SCSO) for PUFA content.

AI and TI are the parameters that refer to the nutritional quality of the oil. The results obtained for AI were low and the same for the all studied oil samples (0.01). Our results for PSO were lower than observed by Rabrenović *et al*. (2021), who found 0.07, 0.08, and 0.07, respectively. For QSO, there is insufficient data in the literature on these quality parameters, so they cannot be compared. The samples had low values of TI, in the range of 0.03 (QSO)-0.02 (PSO and SCSO), while in study of Rabrenović *et al*. (2021) were higher for PSO and SCSO, being 0.15-0.16, and 0.19, respectively. These differences can be explained by different types of PS, different extraction conditions, different storage conditions, and different growth conditions. Low values of these parameters indicate good nutritional quality of oils and their potential for safe use in humans.

Tocopherol composition and content

Tocopherols are considered antioxidants that occur naturally in vegetable oils. These components are mainly responsible for the stability and nutritional value of the oil (Ergönül and Köseoğlu, 2014). Tocopherols occur in four isomeric forms, as α-, β-, γ-, and δ-tocopherols, all of which possess vitamin E activity. The content and composition of tocopherol isomers in the samples are shown in Figure 4.

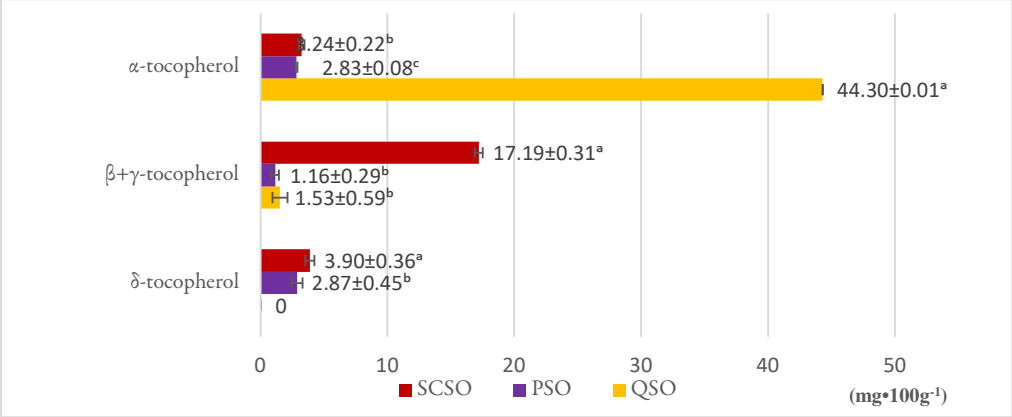

Figure 4. Tocopherol composition and content in oil samples

Results are presented as average ± SD (n=3). Different letters indicate a significant difference according to Tukey's test ($p<0.05$). QSO-quince seed oil; PSO-plum seed oil; SCSO-sour cherry seed oil.

The distribution of the individual tocopherol isomers in the oils studied varied widely. The major tocopherol in QSO was α-tocopherol with a content of 44.30±0.01 mg 100 g^{-1}, followed by β+γ-tocopherol, which is in agreement with the data published by Górnaś *et al.* (2013). In the studied PSO oil, β+γ-tocopherol was present at 1.16±0.29 mg 100 g^{-1} (PSO), while the content of α-tocopherol was 2.83±0.08 mg 100 g^{-1} (PSO). Rabrenović *et al.* (2021) found that the content of β+γ-tocopherol ranged from 48.5 to 57.7 mg 100 g^{-1} and the content of α-tocopherol ranged from 7.0 to 18.6 mg 100 g^{-1} in cold-pressed plum kernel oil, depending on the primary method of plum processing. The value for γ-tocopherol can be significantly higher, as reported by Matthäus and Özcan (2009) for solvent-extracted plum kernel oil, and according to their study, it ranged from 133.1 to 302.1 mg 100 g^{-1}. The low δ-tocopherol content of 2.87±0.45 mg 100 g^{-1} can be compared with the data reported in the studies of Hassanein (1999) and Górnaś *et al.* (2016) with 2.5 mg 100 g^{-1} and 2.0-11.6 mg 100 g^{-1}, respectively.

In SCSO, β+γ-tocopherol dominated with a content of 17.19±0.31 mg 100 g^{-1}, as also reported in the literature (Matthäus and Özcan, 2009; Górnaś *et al.*, 2016), but with an almost tenfold lower content, which may be a consequence of storage of sour cherries in cold storage, the determination method used, different fruit types and other factors. In fact, the studies conducted by Canavar (2015) on peanut kernels showed that prolonged cold storage affects both the ratio of oleic acid to linoleic acid and the tocopherol content, which decreases significantly. With respect to δ-tocopherol, there are contradictions between the data of this study and the literature. For example, the same authors report a significantly higher δ-tocopherol content of 15.1 mg 100 g^{-1} (Rabrenović *et al.*, 2021) and 9.5-18.2 mg 100 g^{-1} (Matthäus and Özcan, 2009) than the 3.90±0.36 mg 100 g^{-1} found in this study.

According to the content of α-tocopherol, QSO is comparable to grape seed oil, whose content of this tocopherol isomer can range from 36 to 309 μg/g oil depending on the grape variety and climate (Lampi and Heinonen, 2009), and to sunflower oil with 32.7-59.0 mg 100 g^{-1} oil (Schwartz *et al.,* 2008). As for the content of gamma-tocopherol, cherry kernel oil stands out, and this content is comparable to that of walnut kernel oil 19.13 mg 100 g^{-1} (Bele *et al.*, 2013).

The differences in the contents of the individual tocopherol isomers found in the literature can be explained by the different varieties of the raw material, the climatic conditions and the process used to obtain the oil.

Phytosterol content

Phytosterols are important components of vegetable oils and contribute to lowering LDL cholesterol levels in blood serum, have anti-cancer properties, and help to improve immunity in humans.

As described in the literature, β-sitosterol is present in most vegetable oils (Yang *et al*., 2019), which was also the case in the studied oils, in which it dominated and accounted for 68.34±2.51% (PSO), 70.12±1.37% (KSO) and 72.98±1.62% (SCSO) of the total phytosterol content (Table 2).

Table 2. Content of phytosterols and squalene (% of total phytosterol content)

Component (%)	QSO	PSO	SCSO
β-Sitosterol	70.12±1.37[a]	68.34±2.51[a]	72.98±1.62[a]
Campesterol	1.20±0.38[c]	3.10±0.53[b]	8.10±0.83[a]
Squalene	0.64±0.08[c]	2.58±0.62[b]	5.50±0.70[a]

Results are presented as average ± SD (n=3). Different letters in the column indicate a significant difference according to Tukey's test ($p<0.05$).
QSO-quince seed oil; PSO-plum seed oil; SCSO-sour cherry seed oil.

These values are generally consistent with those reported in the literature, with the expected variations that, as with tocopherol, are a consequence of variety, climate, oil extraction procedure, and method of determination. For example, Górnaś *et al*. (2016) reported 77-82% content for β-sitosterol in six samples of sour cherry seed oil and 82.9% in Japanese quince oil, and Anwar *et al*. (2014) detected β-sitosterol with 82.9-84.9% content in plum seed oil. The next phytosterol detected in the samples was campesterol with contents of 1.20±0.38, 3.10±0.53, and 8.10±0.83% for QSO, PSO and SCSO, respectively. According to Cusack et al. (2013), a higher ratio of β-sitosterol to campesterol may enhance the lowering of LDL cholesterol, making QSO of particular interest for human dietary supplementation.

The presence of squalene, a triterpene precursor of cholesterol and vitamin D, in their biosynthesis in the human body is interesting. In fact, the richest source of squalene is shark liver, and among vegetable oils it is most abundant in olive and pumpkin oils, so its presence in these oils, is also of interest and importance. The values in the samples studied were 0.64±0.08%, 2.58±0.62% and 5.50±0.70% for QSO, PSO and SCSO, respectively. It is also mentioned in the literature that cherry seed oil is rich in squalene. Gornas *et al*. (2016) determined squalene content of 65.8-102.8 mg 100 g^{-1} oil in the studied samples of sour cherry seed oil, while squalene content in plum seed oil was 25.7-80.4 mg 100 g^{-1} depending on the variety. The results of this study also show that cherry seed oil is a much better source of this triterpene compared to plum and quince seed oil.

Oxidative stability

The oxidative stability of vegetable oils is an important quality parameter and indicates how long the oil can be protected from oxidation. It depends mainly on the composition of the fatty acids and the antioxidant content, but is also influenced by numerous external factors. Figure 5 shows the results for the induction time in hours determined by the Rancimat test for the oils studied. The longer the induction time (h), the better the oxidative stability of the oil and vice versa.

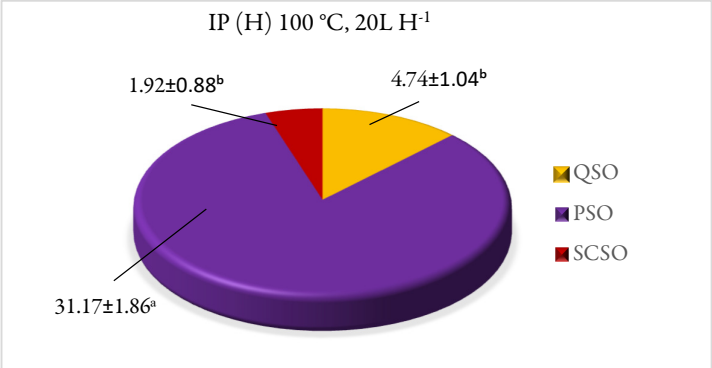

Figure 5. Oxidative stability of oil samples
Results are presented as average ± SD (n=3). Different letters indicate a significant difference according to Tukey's test ($p<0.05$). QSO-quince seed oil; PSO-plum seed oil; SCSO-sour cherry seed oil

As expected, given the fatty acid composition and tocopherol content, PSO showed the best oxidative stability at 31.17 hours. This result is in agreement with the data reported by Rabrenović *et al.* (2021) for the same oil source. They reported an IP (h) of 38.7 for cold-pressed oil from fresh plum seeds. QSO showed a stability of 4.74 h, which is almost double the results reported by Górnaś *et al.* (2013) for Japanese quince seed oil. The value of 1.92 h for SCSO was lower than the value of 3.00 h reported for the same parameter for SCSO (Kazempour-Samak *et al.*, 2021). These differences could be explained by the different origin of the seed, the different storage conditions, the method and conditions used, and other similar factors. However, the value is low, which is probably due to the above-mentioned consequences of cold storage of sour cherries.

Antioxidant activity

The formation of free radicals in vegetable oils cannot be avoided, and it is known that they can cause numerous health problems, including cardiovascular problems, tumours, arthritis and others. Phenolic compounds naturally found in plant seeds play an important role in protecting the body from free radicals. Cold-pressing preserves a significant portion of these compounds in the extracted oil. The quality, stability, and nutritional value of the oil depend on the content of phenolic compounds (Siger *et al.*, 2008). The antioxidant activity of the oils studied in this work is shown in Table 3.

Table 3. Total polyphenol content (TPC) and antioxidant activity (FRP, ABTS and DPPH) of QSO, PSO and SCSO

Samples	TPC (mg GAE 100 mL^{-1})	FRP (mg AA 100 mL^{-1})	ABTS$^{\bullet+}$ (mg Trolox 100 mL^{-1})	DPPH$^{\bullet+}$ (mg Trolox 100 mL^{-1})
QSO	9.03±0.14^{a}	2.90±0.14^{a}	29.77±2.90^{a}	23.56±0.98^{b}
PSO	3.34±0.04^{b}	2.07±0.05^{b}	39.46±4.13^{a}	24.05±3.33^{b}
SCSO	2.28±0.23^{c}	0.73±0.10^{c}	34.71±5.46^{a}	55.65±5.38^{a}

Results are presented as average ± SD (n=3). Different letters in the column indicate a significant difference according to Tukey's test ($p<0.05$).
GAE-galic acid; AA-ascorbic acid; TPC-content of total polyphenols; FRP-ferric reducing power, reducing properties; ABTS$^{\bullet+}$-radical scavenging activity, the ability to collect ABTS$^{\bullet+}$ radicals; DPPH$^{\bullet}$ radical scavenging activity, the ability to collect DPPH$^{\bullet}$ radicals; QSO-quince seed oil; PSO-plum seed oil; SCSO-sour cherry seed oil

The total polyphenol content in the studied oils was 2.28±0.23, 3.34±0.04, and 9.03±0.14 mg GAE 100 mL^{-1} for SCSO, PSO, and QSO, respectively. There are few data in the literature on the total polyphenol content (TPC) of the oils studied. Some data were reported by Wu *et al.* (2011) and Yılmaz and Gökmen (2013) for *Prunus* family oils extracted with different organic solvents, and the yield of TPC was very low. QSO

showed the lowest DPPH activity (23.56±0.98 mg Trolox 100 mL^{-1}), despite the highest content of TPC, which may be due to antagonistic effects between TPC and other chemical compounds in the oil not considered in this study, such as carotenoids, terpenoids etc. In this test, SCSO showed the highest result (55.65±5.38 mg Trolox 100 mL^{-1}). This can be explained by the highest content of γ-tocopherol. The TPC and DPPH values reported by Górnaś *et al*. (2014) for cold-pressed Japanese quince seed oil were significantly higher than the results in this work. QSO showed the highest value for the FRP test and the lowest value for the ABTS test among the samples studied. Literature information on these tests was insufficient. PSO showed the highest ABTS value (39.46±4.13 mg Trolox 100 mL^{-1}), ahead of DPPH (24.05±3.33 mg Trolox 100 mL^{-1}). The antioxidant activity determined by all four assays for PSO and SCSO in this study differed from the values reported by Uluata and Ozdemir (2017). SCSO had the highest DPPH value (55.65±5.38 mg Trolox 100 mL^{-1}), which is explained by the high content of β- and γ-tocopherol. There is insufficient information in the literature about the antioxidant activity of PSO and SCSO.

Conclusions

From the seeds obtained during the processing of the fruits, it is possible to obtain oils that, due to their composition, are very valuable and suitable for both edible and non-edible applications. The oils studied have high content of monounsaturated oleic acid, especially the plum seed oil, which is also reflected in the highest induction time of this oil. Essential linoleic acid was predominant in quince and sour cherry seed oil. Delta-tocopherol was predominant in cherry seed oil, which certainly contributed to the best antioxidant activity of this oil. The results on the composition of fatty acids, tocopherols, sterols, phenolic compounds, and antioxidant potential of oil from quince, sour cherry, and plum seeds may influence their further use. The use of waste raw materials from fruit processing helps to reduce the amount of waste, promotes the circular economy and creates added value for new frameworks.

Authors' Contributions

Conceptualization: MD and BR; Data curation: AĆ and BR; Formal analysis: AĆ and BR; Funding acquisition: AĆ and MFA; Investigation: AĆ and MD; Methodology: MD and BR; Software: AĆ; Supervision: MFA; Writing - original draft: AĆ; Writing - review and editing: BR and AĆ. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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