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Alternative to Conventional Edible Oil Sources: Cold Pressing and Supercritical CO₂ Extraction of Plum (*Prunus domestica* L.) Kernel Seed

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Abstract

Plum (*Prunus domestica* L.) is a fruit widely cultivated across Europe and its processing generates a considerable amount of waste in form of discharged plum kernels. This creates a new opportunity to exploit plum kernels in order to provide an alternative to conventional edible oils. The main aim of this study was to obtain high-quality oil from plum kernel seeds by applying traditional cold pressing (CP) and supercritical carbon dioxide ($ScCO_2$) extraction as a modern technology. The obtained oils were characterized based on the chemical composition of fatty acids and tocopherols. In obtained oils, twelve fatty acids were identified. The oleic acid was the most dominant in both oils (68.66% in oil obtained by $ScCO_2$, 65.86% in oil obtained by CP), followed by linoleic acid (22.24-25.44%). While total tocopherols content in oil obtained by $ScCO_2$ was 4 to 5.8-fold higher than CP. The results proved that the utilization of plum kernel seeds possess high potential as an alternative oil source due a high amount of oleic acid and tocopherols and a low amount of saturated fatty acids and amygdalin.

Keywords: Prunus domestica; supercritical carbon dioxide; cold pressing; tocopherol; fatty acid

1. Introduction

Prunus species comprises 40 different varieties. However, only two species are predominate for industrial application: the European plum (*Prunus domestica* L.) which is hexaploid tree, and the Japanese plum (*Prunus salicina* L.) which is diploid tree. Plum production and processing are widely spread across Europe. Food companies producing dry and canned plum, plum juices and jam; beside, in several European countries plums are used for production of alcoholic beverages. Through processing all are generating large amounts of waste, mostly consist of plum kernel. Apart from being use as biodiesel feed-

stock,³ cheap source of bioactive peptides,⁴ active carbons⁵ or carbonaceous adsorbents,⁶ plum kernel (plum kernel seeds) could be used as a valuable source of oils, which yield can reach above 50%.⁷

Recently, nonconventional oils have gained a lot of attention due to their useful properties. It is already known that some fruit seeds derived from citruses, grapes and watermelon can be used as sources of oils, phenolics and proteins. Citrus seeds contain 20.0–78.9% of oil depending on the species and culitvation conditions. Mathhaus and Ozcan reported that in 17 different citrus seeds the content of oils oleic acid was in the range from 12.8 to 70.1%, linoleic acid from 19.5 to 58.8%, and palmitic acid from 5.1

to 28.3%. Watermelon seeds have an excellent potential for application in food formulations due to the high amount of oil from 50 to 51% and proteins from 32 to 37%. 14 Watermelon seed oil is reported to contain a high content of unsaturated fatty acids from 77 to 82%, a high content of linoleic acid from 59 to 67.5%, and oleic acid from 14 to 18.1%. In addition, grape seeds also contain a considerable amount of oil (14-17%) and a high content of unsaturated acids compared to sunflower and corn oils, which is the main cause for their high commercial interest. Cao and Ito15 reported linoleic acid as the most abundant fatty acid (68.10-78.18%) in grape seed oils. Plum oil is located in the plum kernel seed. According to Velickovic et al. 16 the content of seed in the plum kernel is 136 g/kg for, while content of oil, determined using standard Soxhlet procedure, is 409 g of oil per kg of plum. 16 Same authors identified six main fatty acids in the plum oil: oleic, linoleic, palmitic, stearic and arachidonic acid, out of which oleic (59.5%) and linoleic (27.1%) had the highest percentage.¹⁶

Several different techniques could be applied for separation of oil fraction from the seeds. Among them extraction by organic solvents, supercritical extraction by carbon dioxide (ScCO₂) and cold pressing (CP), are mostly used. Yield of separated oil, oil quality and chemical composition are dependable on applied technique. Advantage of ScCO₂ and CP is the production of safe products, as well as processing in accordance to "green technologies" concept. Therefore, the main aim of this research was to investigate the possibility to obtain high-quality oil from plum kernels seed, using traditional CP and modern technology such as ScCO₂. The oils were characterized based on the chemical composition of fatty acids and tocopherols. The characteristics of oil obtained by ScCO2 were compared to the oil produced by CD. The outcomes may provide a preliminary estimation of the plum kernels seed as an alternative source of edible oil, based upon their fatty acid profile since it is the main quality parameter for edible oils.

2. Materials and Methods

2. 1. Material

Plum kernels were obtained from fruit producer *Plemić komerc doo* (Osečina, Serbia; year of collection 2017). Material was collected in dry condition and pulverized in mill (MRC Sample mill C-SM/450-C, Holon, Israel). Sieve sets (Erweka, Germany) was in employed for determination of the particle size of the grounded material and the average particle size was 0.310 mm. The purity of CO_2 used for extraction was 99.97% (w/w) and purchased from Messer, Osijek, Croatia. For determination of fatty acid composition, Food Industry FAME mix 37 standards (Cat. No. 35077) was purchased from Restek (USA). For determination of tocopherol, α-tocopherol (Dr. Ehrenstorfer Cat No. 17924300), β-tocopherol (Supelco Cat No. 46401-U), γ-tocopherol (Supelco Cat No. 4-7785) and δ-tocopherol (Supelco Cat No. 4-7785)

pelco Cat No. 4-7784) were used. Amygdalin (≥ 99%) standard (A6005-1G) was purchased from Sigma-Aldrich, Cas No. 29883-15-6, USA. All other used chemicals were analytical grade and purchased from J. T. Baker (PA, USA).

2. 2. Cold Pressing of Plum Kernel Oil (PKO)

The cold pressed plum kernel oil was obtained by pressing 1 kg of plum seeds using the following parameters: head presses temperature of 40 °C, frequency of 20 Hz and using a nozzle of ID 6 mm. The pressing of the seeds was performed in a screw expeller SPU 20 (Senta, Serbia) with capacity 20-25 kg/h.

2. 3. Supercritical CO₂ Extraction of Plum Kernel Oil (ScCO₂)

The experiments were carried out using ScCO₂ system explained in detail elsewhere. The extractor vessel was filled with the 100 g of grounded dried plum. The obtained extracts (oil) were collected in glass tubes. Extraction time was 5h. After each 30 minutes, extraction process was paused and the amount of obtained extracts was weighed. The ScCO₂ extraction parameters were as follows: pressure 300 bar, temperature 40 °C, and mass flow rate of 2 kg/h. Conditions in the separator were pressure 15 bar and temperature 25 °C.

2. 4. Analysis of Fatty Acids (FA) Composition

FA methyl esters were prepared according to HRN EN ISO 12966-2:2011 standard method by saponification of glycerides with NaOH in methanol and analysed by gas chromatography carried out using Gas chromatograph 7890B (Agilent Technologies, Lake Forest, USA). Gas chromatography conditions were explained elsewhere. Detained results are expressed as percentage (%) of individual fatty acids to the total fatty acids. The analyses were performed in two replicates.

2. 5. Determination of Content of Tocopherols

Determination of tocopherols $(\alpha, \beta+\gamma, \delta)$ content in PKO obtained by ScCO₂ was performed according to modified HRN EN 12822:2014 standard.²⁰ Analysis was performed using reversed-phase High Performance Liquid Chromatography (HPLC) Infinity 1290 Agilent Technologies (USA) instrument equipped with fluorescence detector (FLD). The analysis was monitored at wavelengths set at 290 and 325 nm, respectively. The instrument was equipped with autosampler G4226A and 1260 FLD G1321C with quaternary pump G4204A. Used column was Zorbax Eclipse XDB, C18 with particle size 5 μ m, and 250 mm long. As a mobile phase was acetonitrile:methanol (50:50) used with gradient run time of 16 minutes, as follow: start flow 2 mL/min and holding for 7 minutes, de-

creasing to 1.5 mL/min. Injection volume was 20 μ L and column temperature was set to 25 °C. Oil for HPLC analysis was prepared as follows. First, certain amount of oil was dissolved in volume of isopropanol providing 88.00–89.00% recovery. Prepared solutions were filtered through 0.2 filters before analysis.

2. 6. Determination of Content of Amygdalin

Cold pressed PKO and $ScCO_2$ extracts (2 g) were weighed into a round-bottom flask, with added ethanol (50 mL). Prepared mixture was boiled under reflux for 120 min. The extracts were filtered and ethanol was completely evaporated under vacuum. Diethyl ether (10 mL) was added to the dried sample and mixture was vortexed about 1 min to precipitate amygdalin. Diethyl ether was evaporated on rotary evaporator and dissolved in water (5 mL). Sample was filtered through a 0.2 μ m PTFE filter before HPLC analysis. ²¹

The method was performed with an Agilent 1290 Infinity I HPLC system equipped with Agilent DAD detector and auto-sampler using (20 μL). The detector was set at 210 nm and the peak areas were integrated automatically, using the Agilent HPLC Data Analysis software Chemstation. Used column for separation was C18 column (4.6 \times 250 mm, 5 μm) at 20 °C. Quantitative analysis was performed with the external standardization by measuring the peak areas. RP-HPLC analysis was performed by isocratic elution with a flow rate of 1.0 mL min $^{-1}$. Used mobile phase was water: methanol (75:25 v/v). The analyses were conducted in two replicates.

3. Results and Discussion

3. 1. Extraction Yield of PKO

The kernels recovered from the plum kernels are a valuable source of oil and significant variations in oil yield

have been reported in different studies.²²⁻²⁴ The causes of variations might be attributed to a different geographic origin, variety, applied extraction technique, etc. Matthäus and Özcan²² determined oil content in *P. domestica* from two difference locations (47.1 and 47.8 g/100 g) using petroleum as extraction solvent. According to Gornas et al.,²³ significant impacts on the PKO yield were related to variety and applied extraction techniques. Gornas et al.²³ reported that the difference between the highest and the lowest level of oil was almost 2.5-fold, while the average content of 28 varieties of two species tested was 38.2% (w/w) where oil extraction was done using *n*-hexane. In a study by Kostic et al.,²⁴ plum kernel oil yield was 35.8%, determined by the Soxhlet extraction, while the PKO yield obtained by pressing was 25.5%, which was 71% of the oil content. Authors concluded that extraction is a more efficient method than pressing since part of oil remains in cake during pressing. However, having in mind environmental and economic disadvantages of organic solvents, pressing is a more appropriate method.²⁴

In this study, efficiency of ScCO₂ as a modern extraction technique and CP technique as a traditional one was compared. Extraction yield obtained with CP was 30.85%, whereas the total extraction yield obtained by ScCO₂ was higher, 38.70%. ScCO₂ extraction kinetics, that is, the extraction yield in function of extraction time, is presented in Figure 1.

3. 1. Composition of Fatty Acids

Seed oils from *Prunus* species have already been reported to possess a highly desirable fatty acid composition with a high content of oleic acid, variable contents of linoleic acid followed with low content of saturated fatty acids. According to fatty acid composition, that may result higher consumer preferences than for olive oil.²² Twelve fatty acids in the kernel oils of plum were identified. The oleic and linoleic acids were predominant in PKO in both cold pressed

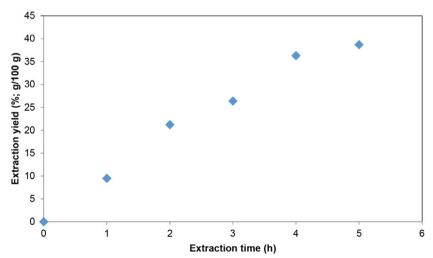


Figure 1. Extraction yield of PKO obtained by ScCO₂

and ScCO2 extracts. These results are consistent with the ones from a published study by Kiralan et al.²⁵ where oleic acid was predominant, followed by linoleic and palmitic acids. Kamel and Kakuda²⁶ also reported oleic and linoleic acids as prevailing fatty acids in peach kernel oil. The oleic acid is 18-carbon monounsaturated fatty acid, essential and highly preferable in human nutrition. It is attributed to have positive effects such as reducing triglycerides, total cholesterol, and glycemic index in human metabolism. Moreover, the increase in oxidation stability in vegetable oil is often assigned to oleic acid presence.²⁷ The PKO is characterized with a high content of oleic acid (68.66% in PKO obtained by ScCO₂, 65.86% in PKO obtained by cold pressing) and a significantly lower amount of saturated fatty acids like palmitic acid and stearic acid (5.80% and 1.92% in PKO obtained by ScCO₂, respectively; 5.79% and 1.62% in PKO obtained by cold pressing, respectively). Similar results were obtained by Kiralan et al.²⁵ where the content of oleic acid was higher (75.43%), the content of palmitic acid was slightly higher (5.83%), and the content of stearic acid was slightly lower (1.35%) in P. domestica oil. The oleic (55-83%) and palmitic (7.5–20%) acids were the prevailing fatty acids in olive oils, followed by stearic acid (0.5–5.0%).²⁸ Detailed fatty acid profile is displayed in Table 2. Moreover, the composition of the obtained fatty acids has a number of similarities with other kernel oil studies. 29,30 However, notable differences were not found in fatty acid profiles of PKO obtained by ScCO2 and CP.

Considering its nutritional value, genus *Prunus* kernels represent an attractive oil for human consumption due to high values of oleic and linoleic acids. In a study by Matthäus and Özcan,²² the mean value for the content of oleic acid was 66.9% and for linoleic acid 22.7% which is in

agreement with our findings. Regarding fatty acid composition, the *Prunus* kernel seed oil could be compared to mid-high-oleic rapeseed oil which does not possess α -linolenic acid which is sensitive to oxidation. It is convenient that the amount of this fatty acid is low (0.09% in PKO obtained by ScCO₂, 0.07% in PKO obtained by CP). This is significant for the stability of PKO, especially if food processing requires heat. Therefore, taking into account the low amount of saturated fatty acids, the high amount of monounsaturated oleic acid, and a desirable fatty acid composition, PKO is highly recommended for human consumption as edible oil. 22

3. 2. Tocopherol Content

Tocopherol comprises several lipophilic phenolic compounds commonly found in edible oils, oil products, fatty fishes, cereals, nuts and other fat-contain products.³¹ This vitamin has 4 tocopherol homologues α -, β -, γ -, and δ -tocopherol, and 4 tocotrienol homologues, α-, β-, γ-, and δ-tocotrienol.³² Primary function of antioxidants and vitamin E is terminating free radicals in vivo. 32 The relative reactivity of α , β , γ , and δ -tocopherol forms against oxygen radicals decreases following the order of $\alpha > \beta = \gamma > \delta$.³³ Therefore, α-tocopherol has the highest affinity to α-tocopherol transferprotein and as the consequence highest bioavailability³² and low rate of metabolism.³⁵ However, the deficiency of tocopherol in diet may cause circulatory disorders and influence on the metabolism pathway in muscles. Since vegetable oils provide humans with a significant part of their daily vitamin E dietary requirements, 36 it was of great importance to determine tocopherols profile for PKOs. Results of quantitative HPLC analysis of tocoph-

Table 2. Fatty acid composition of plum kernels obtained by ScCO₂ and by cold pressing

Fatty acid		Average value for ScCO ₂ -PKO [%]	Average value for CP-PKO [%]
C16:0	Palmitic acid	5.80	5.79
C16:1 (cis-9)	Palmitoleic acid	0.90	0.93
C17:1 (cis-10)	Heptadecenoic acid	0.10	0.11
C18:0	Stearic acid	1.92	1.62
C18:1 (cis-9)	Oleic acid, ω - 9	68.66	65.86
C18:2 (cis-9,12)	Linoleic acid, ω - 6	22.24	25.44
C20:0	Arachidic acid	0.13	0.11
C20:1 (cis-11)	Eicosenoic acid	0.09	0.07
C18:3 (cis-9,12,15)	Linolenic acid, ω - 3	0.09	0.07
C22:0	Behenic acid	0.02	< 0.01
C20:3 (cis-8,11,14)	Eicosatrienoic acid	< 0.01	< 0.01
C22:1 (cis-13)	Erucic acid, ω - 9	0.01	< 0.01
C24:0	Lignoceric acid	0.01	< 0.01
ΣSaturated fatty acids SFA		7.90	7.52
ΣUnsaturated fatty acids UFA		92.10	92.48
ΣMonounsaturated fatty acids MUFA		69.76	66.97
ΣPolyunsaturated fatty acids PUFA		w22.33	25.51

LOD - limit of detection; <0.01

Table 3. Content of tocopherols in PKO

РКО	α-tocopherol (mg/g oil)	β + γ -tocopherol (mg/g of oil)	δ-tocopherol (mg/g of oil)
СР-РКО	0.076	1.22	0.149
ScCO ₂			
after 1h	0.157	2.197	0.354
after 2h	0.092	1.46	0.217
after 3h	0.033	0.921	0.131
after 4h	0.019	0.720	0.096
after 5h	0.0063	0.532	0.067
Total	0.310	5.830	0.865

erols in PKOs, cold pressed or obtained by ScCO₂, are expressed as mg of compound per g of oil (mg/g) in Table 3.

The tocopherol homologues (α , β , γ and δ) were detected in cold pressed PKO, and also in PKO obtained by ScCO₂. Whole ScCO₂ process lasted for 5 hours and oil fractions were collected every hour of the extraction in order to determine at which extraction time the most significant solubility of tocopherols in oil is achieved. Previous researches^{37–40} showed that ScCO₂ extraction can provide satisfactory tocopherol yield from kernels and other plant by-products.

According to Hassanein,41 tocopherol content of PKO (0.71 mg/g) was distinctly higher than that of apricot (0.43 mg/g) and peach (0.52 mg/g) kernel oils, where oil was extracted using chloroform-methanol as a solvent. The oils derived from plum contained 85.5% of γ-tocopherol, apricot oil 93.5% of y-tocopherol and peach 97.7% of tocopherol. However, α -and δ -tocopherols were detected in minor amounts. Beta-tocopherol was not detected in the three above mentioned oils. Previous mentioned three kernel oils showed to be highly resistant to autoxidation due to high content of γ-tocopherol.⁴² In our case, the majority of $\beta+\gamma$ -tocopherols were obtained after two hours of ScCO₂. After 1h of ScCO₂, the amount of α -tocopherol is 2-fold higher than the amount obtained by cold pressing. The similar ratio is noticed after 1h of ScCO₂ in β + γ -tocopherols and δ -tocopherol amounts toward the ones obtained by traditional method. It is evident that the highest solubility of tocopherols was in the first hour of the extraction. Solubility decreases with further extraction, and after 5 hours of extraction, a 25 times lower amount of α-tocopherol was extracted in comparison with the first hour. A similar pattern of results was obtained for apricot kernels seeds in a study Pavlović et al.³⁷ where after 1 hour of ScCO₂ extraction the total content of tocopherols significantly decreased. When comparing the total tocopherols content in PKO obtained by ScCO2 to those obtained with a conventional technique, it must be pointed out that a higher tocopherols content (4 to 5,8-fold) was obtained by applying the modern extraction technique (ScCO₂). Obtained tocopherol values correlate fairly well with Górnaś et al.43 where the extraction of PKO was conducted

with hexane and assisted with ultrasound waves. Similarly to their research, γ-tocopherol was found to be predominant.

3. 3. Amygdalin Content

Stones from the Prunus genus fruits are low-cost and could represent considerable sources of proteins potential sources of peptides with biological activity. However, main restriction to the use of these oil sources is the presence of cyanogenic glycosides such as amygdalin.⁴⁴ Amygdalin is cyanogenic glycoside, commonly present in kernels and seeds of different fruits. 45 This glycoside is potentially toxic in the presence of enzymes (β -glucosidases and α -hydroxynitrilelyases), resulting in the release of hydrogen cyanide.46 On the other hand, amygdalin exhibits many positive biological activities such as the anti-inflammatory and anti-cancer activity. Bolarinwa et al.46 reported that seeds from Rosaceae species, especially from subspecies Pomoideae and Prunoideae contained relatively high amounts (0.1–17.5 mg/g) of amygdalin compared to seeds from non-Rosaceae species (0.01-0.2 mg/g). Senica et al.⁴⁷ also reported big differences in amounts of amygdalin in plum varieties. The lowest amount of amygdalin was in seeds of Valjevka variety and the highest amount of amygdalin was in seeds of Jojo variety. Before industrial application of Rosaceae species, it is recommended to perform a determination of amygdalin.⁴⁸ Garcia et al.⁴⁴ reported amygdalin content of 4.39 mg/g present in the plum seeds, which is 10-fold higher in comparison to amygdalin content (0.41 mg/g) found in our sample. A possible explanation for the significant differences in amygdalin content can be found in different environmental conditions, such as geographical origin, atmospheric conditions, and so on.⁴⁷ Amygdalin content in our plum seed was very low, and in both PKOs is present in traces (Table 4). Moreover, amygdalin content in both oils was significantly lower than in fresh plum kernels. The decrease in amygdalin content could be explained by enzyme degradation of amygdalin into degradation products such as cyanide. Both CP and ScCO₂ methods for obtaining plum kernel seeds oil were performed at low temperature (40 °C), avoiding inactivation of enzymes above 100 °C. With respect to differences in variety and extraction method, enzymatic degradation could be responsible for low amygdalin content. Generally, amygdalin content decreased in processed products for all Rosaceae and non-Rosaceae species.46 However, future

Table 4. Amygdalin content in plum kernels, their oil and extract

Sample	Amygdalin content
Plum kernels	0.4100 mg/g of kernels
CP-PKO	0.0025 mg/g of oil
ScCO ₂ -PKO	0.0022 mg/g of oil

LOD (limit of detection) = 0.00024 mg/g; LOQ (limit of quantitation) = 0.00074 mg/g

work should consider the determination of enzymatic degradation products.

4. Conclusions

Despite the growing number of published papers, the fruit kernels are still considered as non-conventional potential oil sources. In view of the current desire for convenience food such as seedless fruits (citrus, grapes, watermelon, cherry, etc.) there is a tendency of growing kernel waste and further disposal issues. Dealing with the issue of pre-consumer or production food waste will be a crucial action prior to improving productivity and sustainability of the food production system. The high oil content in *Prunus* kernel seeds is comparable to commercial oils seeds such as rapeseed or sunflower seeds. Therefore kernels from genus *Prunus* are highly suitable for commercial oil production. Due to this, the utilization of kernels from this genus seems to be an interesting niche to create an extra value from a by-product.

Fatty acids composition of PKOs obtained by $ScCO_2$ and by cold pressing are similar, however, $ScCO_2$ has shown as more efficient considering notably higher yields of tocopherols, especially α -tocopherol. Additionally, obtained results justify the further processing of plum kernels as by-products of the fruit industry for the production of oil for potential food and pharmaceutical applications.

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Povzetek

Sliva (*Prunus domestica* L.) je pogosto gojena na območju Evrope in procesiranje plodov ustvarja znatne količine odpadnih pešk, ki vsebujejo mehko jedrce. Le-ta lahko predstavljajo alternativni vir jedilnih olj. Cilj te študije je bil pridobitev visoko kvalitetnega olja iz jedrc slivovih pešk z uporabo tradicionalne tehnike hladnega stiskanja (ang. CP) in moderne tehnologije ekstrakcije s superkritičnim ogljikovim dioksidom (ScCO₂). Pridobljena olja smo okaraterizirali na osnovi kemijske sestave maščobnih kislin in tokoferolov. Oleinska kislina je bila prisotna v najvišji koncentraciji v olju pri obeh tehnikah ekstrakcije (68.66 % v olju pridobljenem s ScCO₂, 65.86 % pridobljenem s CP), sledila pa je linolenska kislina (22.24–25.44 %). Celotna količina tokoferolov v olju pa je bila v primeru ekstrakcije s ScCO₂ 4–5.8 krat višja kot pri CP. Rezultati kažejo, da imajo jedrca slivovih pešk velik potencial kot alternativni vir olj z visokim deleže oleinske kisline in tokoferola ter nizkim deležem nasičenih maščobnih kislin in amigdalina.



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