

Original article

Comparative Characteristics of Plum Seed Oil Obtained by Supercritical and Soxhlet Extraction

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SUMMARY

Introduction/Aim. Plums, as one of the oldest types of fruits, are very important in the human diet. The most common varieties of plums cultivated in the territory of Bosnia and Herzegovina are Požegača, Čačanska leptotica, Čačanska rodna and Valjevka. They belong to the group of basic fruits rich in various vitamins and minerals. In addition, plum seed oil is used in the cosmetic industry. This oil is a rich source of biologically active components with proven biological effects – it hydrates the skin and improves its elasticity; the skin easily absorbs it without leaving a greasy film on the surface. The aim of this study was to find the optimal method for isolating fatty oil from the seeds of four types of plums with the best characteristics. Their potential as raw material in the cosmetic industry through the application of supercritical and Soxhlet extraction and their characterization was examined.

Methods. Fatty oils were extracted by conventional Soxhlet extraction with non-polar organic solvent and by supercritical extraction with carbon dioxide. The AOAC 930.15 method was used to determine the moisture content of the plum seeds. The oils were characterized by determining the organoleptic characteristics, as well as the acid, iodine, saponification and peroxide number.

Results. The fattiest oil was obtained from the pits of the Čačanska rodna plum using the both extraction methods, supercritical and Soxhlet extraction. Fatty oil of Požegača had the lowest values of iodine and peroxide number, while the acid and saponification number were the lowest within the oil from Valjevka and Čačanska rodna, respectively.

Conclusion. Both extraction methods showed good extraction yields, with higher extraction capacity using Soxhlet extraction. Čačanska rodna had the highest amount of oil. Fatty oils from all varieties showed excellent characteristics, so the plum kernels can be potentially used for obtaining fatty oil for cosmetic purposes due to its beneficial effect on the skin.

Keywords: plum seed oil, supercritical extraction, Soxhlet extraction

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INTRODUCTION

Plum is one of the oldest type of fruits. It belongs to the same genus (*Prunus* L.) which also includes white plum, almond, peach, apricot, sour cherry, cherry laurel, cherry, and cherry plum. The fruit of these species is drupe. European plum or domestic plum (*Prunus domestica* L.) was created by spontaneous hybridization of blackthorn (*P. spinosa* L.) and wild plum (*P. Cerasifa Ehrh.*) in the forests of the Caucasus. Domestic plum has been cultivated in Europe for more than 2,000 years (1). The most common varieties of plums cultivated in the territory of Bosnia and Herzegovina are:

1. Požegača is included in the group of the best plums in the world. Its disadvantage is its great sensitivity to plum hinge virus and late ripening. It is sensitive to low and high temperatures.

2. Čačanska leptotica is originally from Serbia. It was created at the Fruit Research Institute in Čačak by crossing Wangenheim (Wangenheims Frühzwetsche) and Požegača varieties.

3. Čačanska rodna was created in Serbia by crossing the varieties Stanley and Požegača.

4. Valjevka is produced at the Fruit Research Institute in Čačak.

The domestic plum is a tree that can grow up to 12 m, and its lifespan is about 50 years. The fruit of the domestic plum is a juicy drupe, varying in weight (6.5 to 100.0 g) and shape (ovoid, spherical, pear-shaped), with a lateral furrow (2).

Plums are extremely important in the diet because they have a beneficial effects on the digestive organs and regulate the work of the intestines. They belong to the group of basic fruits rich in various vitamins and minerals (3). Due to the high content of potassium (about 200 mg 100 g/1) and the favorable sodium/potassium ratio, plums are recommended for patients suffering from hypertension (4).

The fruits are most often used for processing, including jam production, canning, drying or baking and are regularly consumed throughout the year. In addition, the kernels of the fruit can be used as a starting material for obtaining fatty oil which can serve as a raw material in the pharmaceutical, food, and cosmetic industries (5, 6). Modern research established that the core contains up to 40 - 50% of oil that can be used for cosmetic purposes due to its regenerating and hydrating properties and beneficial effect on the skin (4, 5). Research interest should be directed towards finding the optimal way of iso-

lating the fatty oil and its characterization, examining the potential biological-pharmacological effects in order to define it as a safe and effective natural raw material (7). In the research conducted by Özcan et al. (8), it was determined that the oil has a low content of free fatty acids and a low value of peroxide number. Also, plum pits are rich in unsaturated fatty acids, the most abundant of which are oleic acid (74.19%) and linoleic acid (19.14%). Further more, it was found that the oil is rich in γ -tocopherol (85.5%) and contains α -tocopherol (11.0%) and δ -tocopherol (3.5%) in smaller quantities, though.

The aim of the work was the isolation of fatty oil from the seeds of four types of plums using supercritical and Soxhlet extraction and their characterization.

MATERIALS AND METHODS

The seeds of four types of plum were used: Valjevka, Čačanska leptotica, Čačanska rodna and Požegača (grown in the village of Borogovo, municipality of Osmaci, Bosnia and Herzegovina).

Determination of moisture content in plum seeds

The AOAC 930.15 method (9) was used to determine the moisture content of the plum seeds. The pitted plums are dried in a draft, at room temperature (22 ± 2 °C) for a period of 30 days. Then they were ground (Bosch electric mill). The method was based on the calculation of the mass loss after drying of the fresh seeds to a constant mass. The moisture content was expressed as percentage % (m/m) according to the following expression:

$$\% \text{ of moisture} = m_1 - m_2 / m_1 \times 100$$

where m_1 - mass of fresh seeds (g); m_2 - mass of dry seeds (g).

Extraction methods

When performing Soxhlet and supercritical extraction process, seeds collected in two consecutive years, 2020 and 2021, were used as plant material.

Supercritical extraction process

Fresh seeds were first dried at a temperature of 40 °C, in a dryer (FD 23, Binder, Germany), until

the constant mass. The seeds were then placed in the SCE extractor. It is the main part of the extraction system Autoclave Engineers SCE Screening System (USA). The dried seeds were first exposed to high pressure, then carbon dioxide was introduced in the system. The system was heated to a temperature of 40 °C. During the extraction process, the carbon dioxide was in a supercritical state, with the critical pressure of 7.386 MPa and temperature of 31.08 °C. After the 2 hours, the system was decompressed and the extraction yield was calculated (10).

Soxhlet extraction process

The Soxhlet extraction of plum kernels was performed using the petroleum ether as extraction solvent and adequate equipment. A certain amount of dried kernels was transferred to a filter paper sleeve, closed with cotton wool and put into the Soxhlet extractor. The extractor was then connected to a condenser and a glass balloon, previously dried for 1 h at 105 °C, cooled in a desiccator for 45 minutes and weighed. Petroleum ether was poured into the extractor - its total volume should not exceed 2/3 of the volume of the balloon and the system was left for 24 hours. After that, the balloon was heated in a water bath, petroleum ether distilled from a laboratory flask, condensed in the condenser and poured over the kernels in the extractor. When the petroleum ether completely filled the overflow tube, it poured into the glass balloon. The Soxhlet extraction process was repeated several times. After that, the petroleum ether was evaporated under reduced pressure using a rotary vacuum evaporator (RV 05 basic, HB4 basic, IKA-Werke, Germany). The obtained extract was dried to the constant mass for 2 h at 30 °C and measured (10, 11).

Determination of extraction yield

The extraction yield, expressed as a percentage (% m/m), was calculated according to the following expression:

$$\% \text{ of extraction yield} = m_1 - m_2 / m_3 \times 100$$

where m_1 is the mass of the balloon with extracted oil; m_2 is the mass of the empty balloon; m_3 is the mass of the plum seeds.

Characterization of fatty oil

The oils obtained from the plum seeds of four types of plum were characterized by determining the organoleptic characteristics (clarity, color and smell) as well as by determining the acid, iodine, saponification, and peroxide number (12).

Statistical analysis

Statistical analyzes of obtained data were performed using SPSS 25.0 software, including One-Way Anova test and Independent samples t-test, with significance level of 0.05.

RESULTS

Moisture content in plum seeds

The examined plum seeds had a very low moisture content (from 3.69% to 4.42%) (Table 1). "Čačanska rodna" plum seeds contained the least amount of moisture (3.69), and "Valjevka" plum seeds contained the most moisture (4.42).

Table 1. Moisture content in ground plum pit kernels

Sample serial number	% of moisture [#]
Požegača	4.14 ± 0.01
Čačanska leptotica	3.99 ± 0.01
Čačanska rodna	3.69 ± 0.04
Valjevka	4.42 ± 0.03

*The results are presented as mean value ± SD (n = 3)

There is statistically significant difference in moisture content values (% of moisture) between all plum pit kernels (Independent samples t-test; sig. < 0.05)

Determination of extraction yield

The oil extraction from the kernel pits of four plums varieties was done in two crops, in two consecutive years (2020 and 2021), using the conventional Soxhlet method and using the supercritical extraction. In both years, the least amount of fatty oil (15.24 and 17.16%) was obtained from the pits of the plum called "Čačanska leptotica" by the supercritical extraction method. In both years, the most fatty oil of

Table 2. Oil yields from plum pits using supercritical extraction (300 bar, 40 °C)

Sample serial number	Extraction yield (%), year 2020.#	Extraction yield (%), year 2021.#
Čačanska lepotica	15.24 ± 0.04	17.16 ± 0.08
Čačanka rodna	26.28 ± 0.02	31.88 ± 0.08
Požegača	22.69 ± 0.06	26.46 ± 0.05
Valjevka	22.24 ± 0.05	27.62 ± 0.09

*The results are presented as mean value ± SD (n = 3).

There is a statistically significant difference between extraction yields of all plum pit kernels using supercritical extraction method from two different years (ANOVA, Independent samples t-test; sig. < 0.05).

Table 3. Oil yields from plum pit kernels using Soxhlet extraction

Sample serial number	Extraction yield %, year 2020#	Extraction yield %, year 2021#
Čačanska lepotica	31.50 ± 0.05	35.93 ± 0.08
Čačanska rodna	52.80 ± 0.08	47.85 ± 0.03
Požegača	40.50 ± 0.05	36.55 ± 0.05
Valjevka	42.80 ± 0.02	47.22 ± 0.08

*The results are presented as mean value ± SD (n=3).

There is a statistically significant difference between extraction yields of all plum pit kernels using Soxhlet extraction from two different years (ANOVA, Independent samples t-test; sig. < 0.05).

Table 4. Comparison of extraction efficiency (yield) of plum pit kernel oils obtained using the Soxhlet method and supercritical extraction method

Sample serial number	Extraction yield using Soxhlet extraction (%)#	Extraction yield using supercritical extraction (%)#
Čačanska lepotica	33.54	16.20
Čačanska rodna	50.32	29.09
Požegača	38.52	24.57
Valjevka	45.01	24.93

*The results are presented as mean value ± SD (n=3).

There is a statistically significant difference between extraction yields of all plum pit kernels using the two different extraction methods - Soxhlet extraction and supercritical extraction method (Independent samples t-test; sig. < 0.05).

the tested samples was obtained from the pits of the "Čačanska rodna" plum by supercritical extraction, 26.28 and 31.88%, respectively (Table 2).

In both years, the least amount of fatty oil was obtained from the plum seeds of the "Čačanska lepotica" using the Soxhlet extraction method (31.50% and 35.93%, respectively), while at the same time, the most fatty oil was obtained from the pits of the "Čačanka rodna" plum using this extraction method among the tested samples, 52.80 and 47.85%, respectively (Table 3). Extraction using the Soxhlet

method obtained significantly higher oil yields from all the tested samples. The order of extraction efficiency, i.e. oil yield, was the same for both types of extractions with the significant difference in values mentioned before (Table 4).

Characterization of plum seeds fatty oil

The oils obtained from the seeds of four varieties of plum were clear, of the light yellow color and characteristic smell, and characterized by deter-

mining the acid, iodine, saponification, and peroxide values. Acid number (I_A) is the number that expresses the amount of potassium hydroxide (in milligrams) needed to neutralize the free acids present in 1 g of oil (12). It indicates the content of free fatty acids in the oil. "Valjevka" plum oils (obtained by both applied methods) had the lowest acid value in the range of 2.11 - 2.15 (yield in the year 2020), that is 2.17 - 2.41 (yield in the year 2021), and the highest value of acid value was found in both oils of "Čačanska rodna" 11.01 - 12.58 (yield 2020.), that is 9.15 - 9.21 (yield in the year 2021). "Požegača" and "Čačanska leptotica" yielded oils with an acid number in the

range 5.41-5.85 (yield in the year 2020), that is 5.01 - 5.46 (yield in the year 2021), (Table 5 and Table 7).

Iodine number indicates the number of grams of iodine that can be attached (bound) to 100 g of oil and indicates the degree of unsaturated fatty acids present in the oil (12). The lowest value of the iodine number was in the oil of "Požegača" (obtained by both applied methods), which indicates the lowest content of unsaturated fatty acids in these oils and, consequently, the best oxidative stability, which was expected. Other oils had very similar iodine number values (Table 5 and Table 7).

Table 5. Acid and iodine number of oils obtained by Soxhlet extraction and supercritical extraction from plum pit kernels (yield 2020)

Sample serial number	Oils obtained by Soxhlet extraction [#]		Oils obtained by supercritical extraction [#]	
	Acid number mg NaOH/1 g of oil	Iodine number	Acid number mg NaOH/1 g of oil	Iodine number
Požegača	5.85 ± 0.05	72.26 ± 0.03	5.56 ± 0.06 ^d	72.05 ± 0.05
Čačanska leptotica	5.61 ± 0.09 ^a	83.57 ± 0.04	5.41 ± 0.09 ^{a,d}	81.55 ± 0.06
Čačanska rodna	12.58 ± 0.02	85.10 ± 0.00 ^c	11.01 ± 0.01	85.15 ± 0.05 ^c
Valjevka	2.11 ± 0.09 ^b	87.11 ± 0.09	2.15 ± 0.05 ^b	86.10 ± 0.01

*The results are presented as mean value ± SD (n = 3).

There is a statistically significant difference between acid number and iodine number values from plum pit kernels oils obtained using the two different extraction methods, except between the values marked with a, b, c and d (ANOVA, Independent samples t-test; sig. < 0.05).

Table 6. Peroxide and saponification number of oils obtained by Soxhlet extraction and supercritical extraction from plum pit kernels (yield 2020)

Sample serial number	Oils obtained by Soxhlet extraction [#]			Oils obtained by supercritical extraction [#]
	Peroxide number	Saponification number	Peroxide number	Saponification number
Požegača	6.76 ± 0.04	205.79 ± 0.02	6.15 ± 0.08	202.45 ± 0.08
Čačanska leptotica	7.18 ± 0.07 ^a	199.00 ± 0.04	7.05 ± 0.02 ^a	201.34 ± 0.07
Čačanska rodna	10.52 ± 0.02	191.14 ± 0.03	9.59 ± 0.04	195.41 ± 0.05
Valjevka	7.93 ± 0.03	206.26 ± 0.06	7.19 ± 0.04	205.71 ± 0.09

*The results are presented as mean value ± SD (n = 3).

There is a statistically significant difference between peroxide number and saponification number values from plum pit kernels oils obtained using the two different extraction methods, except between the values marked with a (ANOVA, Independent samples t-test; sig. < 0.05).

Table 7. Acid and iodine number of oils obtained by Soxhlet extraction and supercritical extraction from plum pit kernels (yield 2021)

Sample serial number	Oils obtained by Soxhlet extraction [#]		Oils obtained by supercritical extraction [#]	
	Acid number mg NaOH/1 g of oil	Iodine number	Acid number mg NaOH/1 g of oil	Iodine number
Požegača	5.15 ± 0.04	72.25 ± 0.09	5.01 ± 0.06	69.21 ± 0.04
Čačanska leptotica	5.46 ± 0.09 ^a	84.06 ± 0.11	5.37 ± 0.05 ^a	83.22 ± 0.00
Čačanska rodna	9.15 ± 0.04 ^b	81.15 ± 0.07	9.21 ± 0.02 ^b	82.13 ± 0.08
Valjevka	2.17 ± 0.04	87.15 ± 0.02	2.41 ± 0.03	85.45 ± 0.06

*The results are presented as mean value ± SD (n = 3).

There is a statistically significant difference between acid number and iodine number values from plum pit kernels oils obtained using the two different extraction methods, except between the values marked with a and b (ANOVA, Independent samples t-test; sig. < 0.05).

Table 8. Peroxide and saponification number of oils obtained by Soxhlet extraction and supercritical extraction from plum pit kernels (yield 2021)

Sample serial number	Oils obtained by Soxhlet extraction [#]		Oils obtained by supercritical extraction [#]	
	Peroxide number	Saponification number	Peroxide number	Saponification number
Požegača	6.09 ± 0.08	201.54 ± 0.04	6.41 ± 0.02	204.05 ± 0.07
Čačanska leptotica	7.05 ± 0.04	200.22 ± 0.01	7.27 ± 0.07	199.61 ± 0.04
Čačanska rodna	9.49 ± 0.06	195.31 ± 0.05	10.79 ± 0.04	197.11 ± 0.01
Valjevka	7.43 ± 0.08 ^a	202.47 ± 0.08	7.57 ± 0.01 ^a	203.25 ± 0.08

*The results are presented as mean value ± SD (n = 3).

There is a statistically significant difference between peroxide number and saponification number values from plum pit kernels oils obtained using the two different extraction methods, except between the values marked with a (ANOVA, Independent samples t-test; sig. < 0.05).

Saponification number expresses the amount of potassium hydroxide (in milligrams) needed to neutralize all (free and bound) acids present in 1 g of oil (12). "Čačanska rodna" plum has an oil characterized by the lowest values of the saponification number 191.14 - 195.41 (yield respectively 2020), that is 195.31 - 197.11 (yield respectively 2021), while the other oils had slightly higher and relatively similar values of this parameter (Table 6 and Table 8).

Peroxide number indicates the amount of hydroperoxides and peroxides present in the oil. The peroxide value is considered an indicator of the initial stage of oil oxidation because the hydroperoxides that are formed are extremely unstable and very quickly break down into the so-called secondary oxidation products (22). "Požegača" produced the oil with the lowest value of peroxide number

6.15 - 6.76 (yield respectively 2020), that is 6.09 - 6.41 (yield respectively 2021), while the "Čačanska rodna" was rich in oil with the highest values of this parameter 9.59 - 10.52 (yield respectively 2020), that is 9.49 - 10.52 (yield respectively 2021). "Čačanska leptotica" and "Valjevka" have oils whose peroxide numbers are between the values of the previously mentioned plum varieties. The low value of the peroxide value of "Požegača" is according with the low peroxide number value, because both parameters indicate good oxidative stability of this oil (Table 6).

DISCUSSION

The extraction of bioactive compounds from plants is the important step in the production of raw material for potential application in the pharmace-

utical and cosmetic industry. The content of bioactive compounds in plants largely depends on the choice of the appropriate extraction method, extraction solvents, plant material, and the mass ratio of the plants and solvents. Vegetable oils, as an important source of liposoluble vitamins, triglycerides, phytosterols, phospholipids, squalene, and essential fatty acids can be isolated from plants. The various ingredients of the oil provide energy to the cells, participate in the synthesis of proteins, protect the tissues, participate in the metabolic processes in the cells, and prevent the occurrence of many diseases. Lipids protect from the harmful effects of the external environment and various pathogens (13 - 16).

Fatty oils can be extracted from plants by Soxhlet extraction (SE) with non-polar organic solvents as one of the most used conventional extraction method. SE is also as a model for comparing new extraction techniques. The duration of this kind of extraction is from several hours to several days, it is performed at an elevated temperature with special equipment. On the other hand, this extraction method has some disadvantages. They are related to the use of large volumes of organic solvents, which are harmful to human health and environment, extraction time is long and there is a possibility of bioactive components degradation due to the various internal and external factors, as high temperatures, light, enzymatic reactions, air (17, 18).

With the aim to overcome those limitations, conventional extraction methods have been replaced by new unconventional methods, such as supercritical extraction (SCE) with carbon dioxide (17, 19). This can be a good alternative to conventional extraction methods with the use of harmful organic solvents because it is an environmentally friendly and technologically advanced. SCE is used in many branches of industry, among others, in the pharmaceutical and cosmetic industry. This extraction meth-

od decreases the use of toxic organic solvents, increases safety and selectivity, with the shorter time of extraction. Also, the separation of the extract from the extraction solvent is facilitated, with no degradation of the active compounds and extract contamination with the impurities from the extraction solvent. On the other hand, the equipment for SCE is very complex and expensive (18, 20, 21).

Significantly higher yields obtained using the Soxhlet method might be a consequence of the structure of the pits and poor diffusion of supercritical carbon dioxide through the plant material. On the other hand, using the Soxhlet method, the organic solvent showed good diffusivity and consequently better extraction efficiency. We assume that additional processing of the pits (keeping them in a chamber under pressure before extraction) or extraction at higher pressures would give better results.

CONCLUSION

Both Soxhlet extraction and supercritical extraction could be used for isolating fatty oils from different plum seeds. Soxhlet extraction showed better extraction yields, with higher extraction capacity for all plum varieties. „Čačanska rodna“ had the highest amount of oil. Fatty oils from all varieties showed good characteristics, where oils from „Požeगाचा“ and „Čačanska rodna“ had slightly better characteristics than other oils. Acid number values (up to 12.58 mg NaOH/1 g of oil) and peroxide number values (up to 10.52) indicated good stability of the obtained oils. Furthermore, iodine number values indicated high concentration of unsaturated fatty acids which are very important for skin care. Accordingly, the plum kernels can be potentially used as raw material for obtaining fatty oil for cosmetic purposes due to its optimal characteristics.

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Komparativne karakteristike ulja semena šljive dobijenih superkritičnom i Soxhletovom ekstrakcijom

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SAŽETAK

Uvod/Cilj. Šljive, kao jedna od najstarijih vrsta voća, veoma su važne u ljudskoj ishrani. Najzastupljenije sorte šljive koje se gaje na teritoriji Bosne i Hercegovine su požegača, čačanska lepatica, čačanska rodna i valjevka. Šljive spadaju u grupu bazičnog voća bogatog vitaminima i mineralima. Pored toga, koristi se i seme šljive kao izvor ulja koje nalazi primenu u kozmetičkoj industriji. Ovo ulje je bogat izvor biološki aktivnih komponenata sa dokazanim biološkim delovanjem – hidrira kožu i poboljšava njenu elastičnost; koža ga lako upija, a na njenoj površini ne ostavlja mastan film. Cilj ovog rada bilo je pronalaženje optimalnog načina izolovanja masnog ulja semena četiri vrste šljiva najboljih karakteristika. Ispitivani su potencijali sirovog materijala u kozmetičkoj industriji primenom superkritične i Soxhletove ekstrakcije, kao i njihova karakterizacija.

Metode. Korišćeno je seme četiri vrste šljive: valjevka, čačanska lepatica, čačanska rodna i požegača (gajenih u selu Borogovo, opština Osmaci, Bosna i Hercegovina). Za određivanje sadržaja vlage u semenu šljive korišćena je metoda AOAC 930.15. Masna ulja ekstrahovana su konvencionalnom Soxhletovom ekstrakcijom nepolarnim organskim rastvaračem i superkritičnom ekstrakcijom ugljen-dioksidom. Ulja su okarakterisana određivanjem organoleptičkih karakteristika, kao i kiselinskog, jednog, saponifikacionog i peroksidnog broja.

Rezultati. Superkritičnom ekstrakcijom izolovane su manje količine ulja iz semena šljiva nego primenom konvencionalne Soxhletove ekstrakcije (kod semena sve četiri vrste šljive). Najveći sadržaj ulja dobijen je iz semena šljive čačanska rodna (primenom obeju metoda ekstrakcije). Najniže vrednosti jednog i peroksidnog broja imalo je masno ulje požegače, te ga možemo smatrati najstabilnijim. Kiselinski i saponifikacioni broj bili su najniži kod ulja dobijenog iz semena valjevke i čačanske rodne.

Zaključak. Obe metode ekstrakcije pokazale su dobre prinose ekstrakcije, sa većim kapacitetom ekstrakcije primenom Soxhletovog aparata. Najveću količinu ulja imala je čačanska rodna, a hemijski najstabilnije bilo je ulje semena valjevke. Masna ulja svih sorti mogu se koristiti u kozmetičke svrhe.

Ključne reči: ulje semena šljive, superkritična ekstrakcija, Soxhletova ekstrakcija