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# XXV SAVETOVANJE O BIOTEHNOLOGIJI

sa međunarodnim učešćem

- ZBORNIK RADOVA 2 -



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Čačak, 13 - 14. mart 2020. godine

# **XXV SAVETOVANJE O BIOTEHNOLOGIJI**

**sa međunarodnim učešćem**

**- Zbornik radova 2 -**

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## PREDGOVOR

Promene koje se ubrzano dešavaju na globalnom i lokalnom nivou, od naučnih, klimatskih, ekonomskih, pa do političkih, podstiču potrebu da proučimo njihov uticaj na živi svet i na jednu od najvažnijih ljudskih delatnosti - proizvodnju hrane.

Naša poljoprivreda, selo, poljoprivredni proizvođači nisu danas to što su bili pre trideset ili četrdeset godina, srpsko selo se danas više nego ikad ubrzano i u hodu menja. Poljoprivredna nauka mora preuzeti deo odgovornosti u pogledu proizvodnje dovoljne količine kvalitetne hrane za ljudsku ishranu, jer prolaze vremena kada se za svaku lošu žetvu traže opravdanja u klimi.

S' ciljem da budemo u toku aktuelnih zbivanja, kao i da sami svojim rezultatima utičemo na razvoj poljoprivrede i na delatnosti koje je prate, Agronomski fakultet u Čačku, pored edukacije studenata, redovno, godišnje, organizuje i Savetovanje o biotehnologiji, ovaj put, jubilarno, dvadeset peto po redu.

Osnovni cilj Savetovanja je upoznavanje šire naučne i stručne javnosti sa rezultatima najnovijih naučnih istraživanja, domaćih i inostranih naučnika iz oblasti osnovne poljoprivredne proizvodnje i prerade, kao i zaštite životne sredine. Na taj način Fakultet nastoji da omogući direktan prenos naučnih rezultata široj proizvodnoj praksi, pa pored naučnih radnika, agronoma, tehnologa, na ovogodišnjem Savetovanju biće i značajan broj poljoprivrednih proizvođača, stručnih savetodavaca, nastavnika, itd.

U Zborniku radova jubilarnog XXV Savetovanja o biotehnologiji sa međunarodnim učešćem, predstavljeno je ukupno 86 radova iz oblasti Ratarstva, povrtarstva i krmnog bilja, Voćarstva i vinogradarstva, Zootehnike, Zaštite bilja, proizvoda i životne sredine i Prehrambene tehnologije.

Pokrovitelj jubilarnog XXV Savetovanja o biotehnologiji sa međunarodnim učešćem je Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije, a materijalnu i organizacionu podršku su nam pružili grad Čačak, privrednici, dugogodišnji prijatelji Agronomskog fakulteta, kojima se i ovim putem zahvaljujemo.

U Čačku, marta 2020. godine

Programski i Organizacioni odbor  
XXV Savetovanja o biotehnologiji

## PRELIMINARY ASSESSMENT OF FATTY ACID PROFILE IN ROSEHIP SEEDS: APPLICATION OF ULTRASOUND-ASSISTED EXTRACTION

*Dušan Vasić<sup>1</sup>, Dragana Paunović, Bojana Špirović Trifunović, Jelena Miladinović, Lazar Vujošević, Ilinka Pećinar, Jelena Popović-Đorđević<sup>1</sup>*

**Abstract:** Rosehip seeds represent waste material which is obtained during the production of jams, marmalades, juices, teas, etc. Two methods were used for the extraction of fatty acids from rosehip seeds: ultrasound-assisted extraction combined with organic solvent extraction (UAE/OSCE) and organic solvent conventional extraction (OSCE). Different solvent-to-sample ratio was used. The identification and quantitation of fatty acids was done by gas chromatography with a flame ionization detector (GC/FID). Applied methods yielded different types of fatty acids, with stearic acid and linoleic acid, as the most abundant ones.

**Key words:** rosehip seed, fatty acids, ultrasound-assisted extraction, GC-FID

### Introduction

Dog rose (*Rosa canina* L.) is widespread in almost all of Europe, western and northern Asia and Africa (Nowak, 2005). In Serbia it can be found on Fruška Gora, Kopaonik, Avala, Suva Planina, Stara Planina, etc. (Mratinić and Kojić, 1998). The pseudo-fruit of the dog rose is known as rosehip, which can be used as a component of functional food, as well as in cosmetics and pharmaceuticals, because it is rich in bioactive compounds, especially antioxidants and essential elements (Ercisli et al., 2007; Ilyasoğlu, 2014; Paunović et al., 2019). Rosehip fruits contain 30-35% of seeds, and the content of oil in seeds may vary from 3-7 % depending on applied extraction method (Zlatanov, 1999; Dabrowska et al., 2019).

The ultrasonic-assisted (or ultrasound-assisted) extraction technique (UAE) was used as a supplementary technique for extracting oils from the seeds. The advantages of this extraction technique over other methods are: increased efficiency, reduction of the extraction time period and often better quality of the extract (Cravotto et al., 2008). The ultrasonic-assisted extraction does not require high temperatures; on the contrary, this technique can be carried out at low temperature which contributes to the preservation of the extracts bioactive compounds (Yuting et al., 2013). To obtain maximum results this method requires optimization of UAE parameters (ultrasonic power, extraction time and temperature, as well as the ratio of dissolvent volume to seed weight) (Yuting et al., 2013).

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<sup>1</sup>University of Belgrade-Faculty of Agriculture, Belgrade, Serbia ([jelenadij@agrif.bg.ac.rs](mailto:jelenadij@agrif.bg.ac.rs) ; [vasic449@gmail.com](mailto:vasic449@gmail.com))

The aim of this work was the extraction of fatty acids from rosehip seeds using different methods, and their identification by gas chromatography (GC) with flame ionization detector (GC-FID) after derivatization with  $\text{BF}_3/\text{MeOH}$  reagent.

## Material and methods

Rosehip (*Rosa canina* L.) originated from the vicinity of Čačak city (Republic of Serbia) were purchased at the local market in the autumn of 2018.

Prior to analyzing fatty acid (FA) content, rosehip samples were prepared by the following procedure: the seeds were first separated from the mesocarp of the fruit. Further, seeds were air-dried and then ground using a blender. Two methods for the extraction of fatty acids from the rosehip seeds have been used: ultrasound-assisted extraction combined with organic solvent extraction (UAE/OSCE) and organic solvent conventional extraction (OSCE). Samples of ground seeds were weighed on an analytical balance into glass vials and appropriate volumes of solvent (*n*-heptane) were added. Solvent-to-sample ratio (S/S) was 3:1, 4:1 and 5:1 for UAE/OSCE (samples 1-3) and 3:1 for OSCE (sample 4). Ultrasound was applied for 1.5 hours. After that period, samples were subjected to OSCE for additional 68 hours. OSCE was performed within 70 hours (with occasional shaking). All extractions were performed at room temperature ( $\sim 23$  °C).

After solvent evaporation 1 ml of hexane was added in lipid fraction for its dissolution and better efficiency of derivatization with 14 % Boron trifluoride methanol reagent. To complete derivatization reaction, mixture was heated at 100 °C for one hour. Fatty acid methyl esters (FAMES) were extracted in hexane phase after addition of water, and then analyzed with GC-FID (Barać et al., 2018).

Fatty acid content calculated as mg/g lipid and expressed as a relative amount of total fatty acids expressed in percent (%) was identified by comparing the retention times with the peaks of the analytical standard acid mix containing 37 acids (Supelco, Bellefonte, SAD).

## Results and discussion

The results obtained for fatty acid (FA) composition of rosehip seeds lipid fraction by the application of two methods are presented in Table 1. Three samples (1-3) were subjected to ultrasound-assisted extraction combined with organic solvent extraction (UAE/OSCE) while one sample (4) was extracted by conventional organic solvent method (OSCE). The most abundant FA in studied rosehip seed oil samples was stearic acid (48.11%), followed by linoleic acid (35.38%), palmitoleic acid (33.78%) and eicosadienoic acid (30.57%). Among detected fatty acids only four were identified as saturated (stearic, arachidic, heneicosanoic and behenic acid). The other fatty acids were detected and identified as monounsaturated and polyunsaturated fatty acids (Table 1).

Table 1. Content of fatty acids in samples obtained by different extraction methods  
 Tabela 1. Sadržaj masnih kiselina u uzorcima dobijen različitim metodama ekstrakcije

Extraction method <i>Metod ekstrakcije</i>		UAE/OSCE			OSCE
Sample <i>Uzorak</i>		1	2	3	4
	Fatty acid <i>Masna kiselina</i>	Content of FA (%) <i>Sadržaj MK (%)</i>			
C14:1	miristoleic	/*	/	9.60	/
C16:1	palmitoleic	18.25	9.59	31.59	33.78
C17:1	<i>cis</i> -10-heptadecanoic	/	/	7.29	/
C18	stearic	/	/	2.30	48.11
C18:1n9c	oleic	9.3	/	11.53	18.12
C18:2n6c	linoleic	13.88	35.38	/	/
C18:3n3	$\alpha$ -linoleic	/	/	7.12	/
C20	arachidic	7.58	28.21	/	
C20:2	eicosadienoic acid	/	/	30.57	/
C20:3n6	<i>cis</i> -8,11,14-eicosatrienoic	23.12	/	/	/
C20:3n3	<i>cis</i> -11,14,17-eicosatrienoic	14.69	/	/	/
C20:4n6	arachidonic	13.17	/	/	/
C21	heneicosanoic	/	21.43	/	/
C22	behenic	/	5.39	/	/
$\Sigma$ SFA		7.58	55.03	2.30	48.11
$\Sigma$ UFA		92.42	44.97	97.70	51.89
$\Sigma$ MUFA		18.25	9.59	60.01	51.89
$\Sigma$ PUFA		74.17	35.38	37.69	0

\* / - not detected;  $\Sigma$ SFA - sum of saturated fatty acids;  $\Sigma$ UFA - sum of unsaturated fatty acids;  $\Sigma$ MUFA - sum of monounsaturated fatty acids;  $\Sigma$ PUFA - sum of polyunsaturated fatty acids

\* / - nije detektovano;  $\Sigma$ SFA - ukupno zasićenih masnih kiselina;  $\Sigma$ UFA - ukupno nezasićenih masnih kiselina;  $\Sigma$ MUFA - ukupno mononezasićenih masnih kiselina;  $\Sigma$ PUFA - ukupno polinezasićenih masnih kiselina

Samples 1 and 4 had the same solvent-to-sample ratio (S/S- 3/1), but it is evident that the method of extraction affected on the number of detected fatty acids. The most abundant FA in sample 1 was *cis*-8,11,14-eicosatrienoic, while the dominant FA in sample 4 was stearic acid. Unlike other long-chain saturated fatty

acids, stearic acid has no effect on cholesterol lipoprotein concentration (Yu et al., 1995). Palmitoleic acid is an omega-7 UFA and it is a common constituent of human adipose tissue glycerides, and it has been shown to have an antithrombotic effect meaning it can prevent stroke (Stedman, 1995; Parveez et al., 2012; Orsavova et al., 2016). Palmitoleic acid was detected in all samples in this experiment. Oleic acid, detected in samples 1, 3 and 4, is an omega-9 fatty acid and it is one of the most widespread fatty acids in nature (Stedman, 1995). Linoleic acid is one of two essential fatty acids for humans and have a very important role in metabolism (MacDonald, 2000). In our study this FA detected in two samples (1 and 2).

Similar research was conducted by Nowak (2005), Machmudah et al. (2007) and Kazaz et al. (2009), but with use of different extraction methods. In research of Nowak (2005) it was found that the predominant acid in rosehip seeds was oleic, while saturated acids palmitic, stearic and *cis*-11,14-eicosadienoic were present in the traces. Machmudah et al. (2007) found  $\gamma$ -linoleic and stearic acid in rosehip seeds. Kazaz et al. (2009) also analyzed rosehip seed and as the dominant acids they obtained oleic and  $\gamma$ -linoleic acid, while stearic acid was found in a small amount. Obtained results of Zlatanov (1999) indicated that the highest concentration of oleic acid in rosehip seeds was in agreement with the samples of other authors (Nowak, 2005; Ilyasoğlu, 2017). Javanmard et al. (2018) and Ilyasoğlu (2017) found equal amounts of oleic and  $\gamma$ -linoleic acid.  $\gamma$ -Linoleic acid is an isomer of  $\alpha$ -linoleic acid and belongs to the group of omega-6 fatty acids. In our study  $\alpha$ -linoleic was detected only in sample 3. Differences in the composition and amount of fatty acids in rosehip seed oil can be the result of different climatic, soil, environmental and genetic factors.

## Conclusion

According to the obtained results, applied extraction methods, as well as the sample-to-solvent ratio had influence on the composition of fatty acids and their relative amounts. Among saturated and unsaturated FA, stearic acid and linoleic acid, respectively, were the most abundant ones. The results obtained in this experiment open the possibility for further studies in this topic.

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## PRELIMINARNO ISPITIVANJE SASTAVA MASNIH KISELINA U SEMENKAMA ŠIPURKA: PRIMENA ULTRAZVUČNE EKSTRAKCIJE

*Dušan Vasić<sup>1</sup>, Dragana Paunović, Bojana Špirović Trifunović, Jelena Miladinović, Lazar Vujošević, Ilinka Pećinar, Jelena Popović-Đorđević<sup>1</sup>*

**Izvod:** Semenke šipurka predstavljaju otpadni materijal, bogat uljem, koji nastaje tokom proizvodnje džemova, marmelada, sokova, čajeva itd. Za ekstrakciju masnih kiselina iz semenki šipurka korišćene su dve metode: ultrazvučna ekstrakcija u kombinaciji sa ekstrakcijom organskim rastvaračem (UZE/KEOR) i konvencionalna ekstrakcija organskim rastvaračem (KEOR). Korišćeni su različiti odnosi zapremine rastvarača i mase uzoraka. Identifikacija i kvantifikacija masnih kiselina izvršena je metodom gasne hromatografije sa plameno-jonizujućim detektorom (GC/FID). Primenjenim ekstrakcionim metodama dobijene su različite masne kiseline, od kojih su stearinska i linolna bile najzastupljenije.

**Ključne reči:** semenke šipurka, masne kiseline, ultrazvučna ekstrakcija, GC-FID

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<sup>1</sup>Univerzitet u Beogradu-Poljoprivredni fakultet, Nemanjina 6, Beograd, Srbija  
([jelenadj@agrif.bg.ac.rs](mailto:jelenadj@agrif.bg.ac.rs); [vasic449@gmail.com](mailto:vasic449@gmail.com));

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