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Chemical characterization of *Rosa canina* L. rosehip seed: application of Raman spectroscopy and gas chromatography

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Abstract: Rosehip seeds represent the food industry waste material, in production of marmalade, jam, beverages, jelly, syrup, tea, etc. Agri-food wastes are rich in bioactive compounds and nutrients that can add value to different fields of agriculture and food production. The aim of this study was to assess the chemical composition of seed from *Rosa canina* L. hips, with the focus on seed oil fatty acid profile. In this respect, analytical methods *in situ* Raman spectroscopy (RS) and gas chromatography (GC) were used. Fatty acids in form of methyl esters (FAMEs) were analyzed by gas chromatography with a flame ionization detector (GC/FID). Raman spectra showed the presence of lipids, fatty acids, poliphenolics and saccharides (including cellulose) as the predominant classes of compounds in seeds. Bands at 1266, 1328, 1369 and 1655 cm^{-1}, were associated to lipids and unsaturated fatty acids (UFAs). The spectra also indicated *cis* isomers in the lipid fraction. Seeds contained 5.6 % of oil, and GC analysis confirmed the presence of UFAs, linoleic acid (ω-6) and α-linolenic acid (ω-3) (29.72 and 4.20%, respectively). Raman spectroscopy was applied as the fast and nondestructive analytical method for the chemical evaluation of rosehip seeds. Results of GC analysis showed that rosehip seeds are good source of nutritionally valuable fatty acids that might be utilized in products specified as functional food.

Keywords: rosehip seed; waste material; essential fatty acids; Raman spectroscopy; gas chromatography

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1. Introduction

Dog rose (*Rosa canina* L.), the well-known and traditionally used European species has been recently considered as a complex of species (the aggregate) due to genetic and related morphological polymorphism [1]. Due to its nutritional value and sensory properties, as well as the abundance of bioactive compounds, rosehip takes a significant place in the human diet and food industry [2,3]. Rosehip fruits contain about 30–35% of seeds [4], which are considered as the waste material in the in production of marmalade, jam, beverages, jelly, syrup, tea, etc. In the recent years due to their specific fatty acid composition rosehip seeds have been used in the cosmetic and pharmaceutical industries [5]. They are good source of linoleic, linolenic, palmitic and stearic acid. The predominant are linoleic and α-linolenic which are essential fatty acids that have a very important role in metabolism [6-8]. Fatty acids extracted from seeds also show significant antibacterial, antioxidant and anti-inflammatory activity [9].

Conventional methods for chemical composition analysis of fruits and seeds (HPLC, TLC, UV/visible spectrophotometry, etc.) usually require long procedures of standardization; involve time consuming extraction steps and expensive chemicals [10]. On the other hand, *in situ* analysis by Raman spectroscopy as rapid and non-destructive method may provide chemical and structural information with minimum requirements for sample pre-processing [11,12].

The aim of this study was to assess the chemical composition of seed from *Rosa canina* L. hips using Raman spectroscopy and gas chromatography, with the focus on seed oil fatty acid composition.

2. Material and methods

2.1. Plant material

About 50 rosehip (*Rosa canina* L.) specimens (ripened fruits) were collected from the rural area near Čačak city (locality Gornja Trnava, Moravica district, Central Serbia) in the autumn of 2018. Plant sample was deposited in the Herbarium of the Faculty of Agriculture, Belgrade-Zemun, Serbia. The collected rosehips were washed by tap water and dried at room temperature. Fleshy fruit parts (hypathium) and seeds (Figure 1a and b) were first separated, and then placed at low temperature (ca. -18 °C) until the Raman spectroscopy analyses to prevent damage of the chemical composition of samples. Additionally, for the purpose of gas chromatography analysis (Figure 1c) seeds were ground before freezing using a blender (BOSCH MKM6000, 180 W, Slovenia).

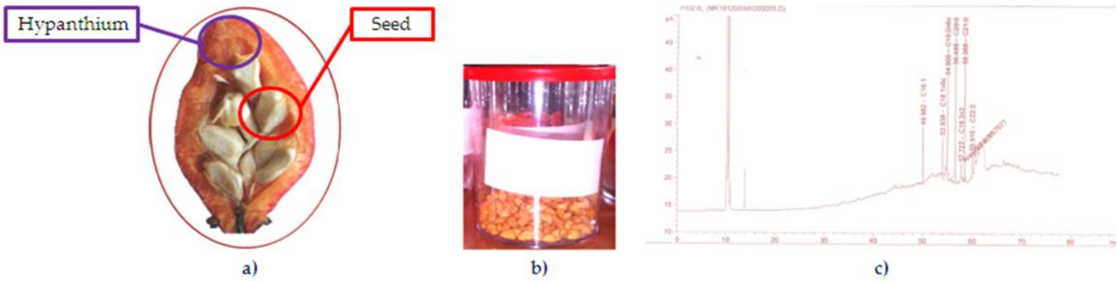


Figure 1. Rosehip hypathium and seed (a), separated seeds (b) and the chromatogram of seed FAs profile (c).

2.2. *Raman Instrumentation*

Raman microspectroscopy was focusing on direct analysis of seeds which were longitudinally cut at room temperature prior to analysis. Spectra were recorded in the range 200-1800 cm^{-1}, using XploRA Raman spectrometer (Horiba Jobin Yvon) following the procedure described in literature [12]. The spectra preprocessing was realized using Spectragryph software, version 1.2.13 [13].

2.3. Extraction of oil fraction and *fatty acids analysis*

Prior to fatty acids (FAs) analysis, the ground seeds were defrosted. About ~2.5 g was weighted on an analytical balance transfered into glass vials and then 7 ml of *n*-heptane was added in order to extract FAs. The extraction of fatty acids from the rosehip seeds was performed at room temperature (~23 °C) using ultrasound-assisted extraction (UAE) for 1.5 hour on an ultrasound instrument (Baku BK-3A, China) with a volume of 1 L, a frequency of 40 kHz and an input power of 30 W. After the extraction was complete sample was filtered through quantitative filter paper (pore size 2-4 μm), and the solvent was evaporated. Mass of oil fraction was measured after the solvent removal and expressed in percent (%).

After solvent removal oil fraction was derivatized with BF_3/MeOH reagent to convert FAs into fatty acids methyl esters (FAMEs) which were analyzed by gas chromatography with a flame ionization detector (GC-FID) using Agilent Technologies 6890 (USA) instrument as described in literature [14]. The content of FAs was identified by comparing the retention times with the peaks of the analytical standard acid mix containing 37 FAMEs (Supelco, Bellefonte, SAD).

3. Results and discussion

3.1. Raman spectroscopy analysis

With Raman spectroscopy we demonstrate the fast and nondestructive feature of the method on seeds of rosehip. Chemical composition of *Rosa* sp. seeds was evaluated based on bands recorded in the region 200-1800 cm^{-1} (Figure 2).

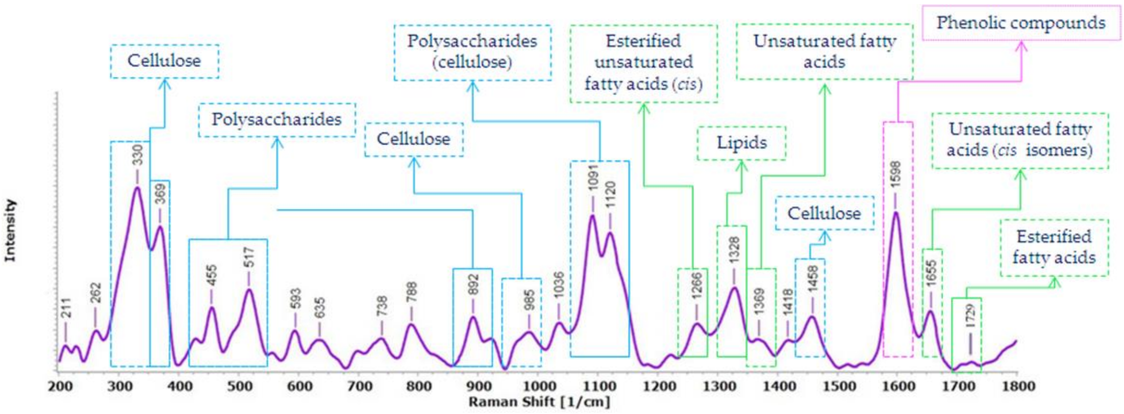


Figure 2. Average Raman spectrum of rosehip seeds and band assignments (200 to 1800 cm^{-1}).

The fingerprint region of Raman spectra in *Rosa canina* seed includes essential bands which correlate with the most important parts of the fatty acids molecular structure [15], also the region is well known to characterize the unsaturation level of the fatty acid chain [16]. The predominant fatty acids detected in the rosehip seeds are unsaturated acids (UFAs) (linolenic, linoleic and oleic), with the highest percentage of linoleic acid [2] and in the lower percentage of saturated fatty acids (SFAs) (palmitic and stearic) [9]. In the Raman spectra of linolenic, linoleic and oleic acid, there are three or two broad C=C bonds with higher wavenumbers [17]. These acids manly differ in the position of the double bond, consequently their Raman spectra are highly similar [18]. The bands at 1655 and 1266 cm^{-1}, observed in the seed spectrum (Figure 2), are related to the presence of unsaturated fats and can be assigned to the *cis* stretching vibration of C=C and the bending of C-H, respectively [11]. All involving the unsaturation moieties of unsaturated fatty acids *cis* isomers, which relative intensity is in accordance with the degree of saturation of the fatty acid in the lipid, especially in a case of the band at 1655 cm^{-1} [17]. The lowest intensity band at 1729 cm^{-1} (Figure 2) was assigned to the stretching of C=O from triacylglycerol structure that was present in all Raman spectra of different plant oil samples [17].

The presence of phenolic compounds in Rosa seed was indicated by higher intensity signals at 1598 and 369 cm^{-1} (Figure 2), that primary originate from lignin of seed cell wall compounds [19,20]. Additionally, the higher intensity bands such as 1091 and 1120 cm^{-1} in the spectrum (Figure 2), could be assigned to polysaccharides due to C-O-C, and 1458 cm^{-1} due to C-O-H stretching vibrations of carbohydrates [10,11].

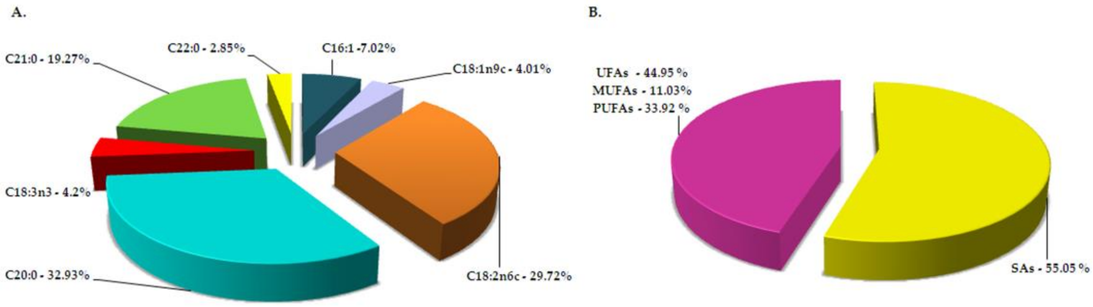
3.2. Fatty acid content

Lipids are considered one of the most fundamental constituents in human nutrition. Fatty acids are major constituents of lipids, and essential fatty acids (EFAs) such as ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) have to be acquired from a diet. Also these FAs have been considered as functional food and nutraceuticals. Many researchs have delineated their significant roles in many biochemical processes, resulting in health promotion activities [21,22]. Most naturally occurring UFAs have *cis* configuration, while

FAs with *trans* configuration occure in products as a result of technology processing (i.e. hydrogenation) [21].

The content of oil in rosehip seeds is low, up to 15 %. The extraction procedure affects the oil yield and the modern methods usually provide higher yields comapred to the Soxhlet extraction [5,22]. In this study, the yield of seed lipid fraction obtained by the application of ultrasound-assisted extraction (UAE) was 5.6 % (0.14 g per 2.5 g of seeds), which is in line with literature data for other extraction methods [5,22]. The modern extraction methods such as ultrasound, microwave and subcritical sub-critical fluid extraction, are utilized for obtaining higher quality oils [5].

Fatty acid content was calculated as mg/g lipid and expressed as a relative amount in percent (%) of total FAs. Results revealed that the most abundant FA in studied rosehip seed oil sample was arachidic (32.93%), followed by linoleic acid (29.72%), heneicosanoic (19.27%), palmitoleic acid (7.02%), α-linolenic acid (4.20%), oleic acid (4.01%) and behenic acid (2.85%) (Figure 3).



(**a**) (b)

Figure 3. Fatty acids aboundance (a) and the saturated and unsaturated FAs ratio (b) in rosehip seed.

Among detected FAs, two acids belong to omega-7 (palmitoleic acid) and omega-9 (oleic acid) monounsaturated fatty acids (MUFAs) whereas linoleic acid and α-linolenic acid are ω-6 and ω-3 PUFAs, respectively. The relative content of SFAs were somewhat higher compared to UFAs (Figure 3b). The unusually high percentage of arachidic acid might be a consequence of storage conditions as well as the applied extraction method.

Results obtained in this study for UFAs are in line with literature; as it is reported that the most abundant ones were linoleic, oleic, linolenic and α-linolenic in seeds of rosehip (*R. canina L.*) originating from different regions of the World. On the other hand, variability in qualitative and quantitative composition of FAs in seeds is well documented [2,4,6,7,23-25]. Data about chemical composition and FAs profiles of rosehip seeds could indicate that differences may result from the influence of numerous factors such as climatic, environmental, genetic, etc.

Conclusion

This study confirmed the successful application of Raman spectroscopy in the detection of lipids and fatty acids in seed storage reserves of rosehips in a straightforward and fast manner. The bands in the spectrum clearly indicated the presence of *cis* UFAs, and GC analysis confirmed that linoleic acid was the most abundant one. Raman spectroscopic analysis also detected phenolic compounds and polysaccharides in seeds.

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