Supercritical extraction from *Rosa canina* L. fruits: fatty acids composition and biological activities

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The supercritical fluid extraction from pulp of *Rosa canina* L. fruits, using CO_2 as a solvent, is presented in this study. The extraction experiment was carried out at pressures of 300 bar and a temperature of 40°C, SFE[300:40]. The extract yield was 0.3% for the weight of the charge. The extraction and saponification processes produced a fraction mainly formed by free fatty acids, FA, determined by HPLC-DAD and GC-FID analyses. Pulp extract was characterised by a high level of linolenic acid, 18:3 n-3, (28.37% of total FA); linoleic acid, 18:2 n-6, (26.74%); palmitic acid, 16:0, (18.20%); and oleic acid, 18:1 n-9, (15.74%). Followed by low amounts of stearic acid, 18:0, palmitoleic acid, 16:1 n-7, lauric acid, 12:0, and myristic acid, 14:0.

The amounts of the main unsaturated fatty acids, UFA, in SFE[300:40], determined by HPLC analysis, were 121.43 ± 3.21 mg/g, 102.16 ± 2.84 mg/g, and 49.95 ± 2.75 mg/g of extract for 18:3 n-3, 18:2 n-6 and 18:1 n-9, respectively. Interestingly, the sample was characterised by a high proportion of polyunsaturated fatty acids, PUFA, and the ratio value of UFA to SFA, saturated fatty acids, was 2.8.

The quality of the SFE[300:40] extract, in terms of its chemical composition, was compared with that obtained using n-hexane in a Soxhlet apparatus, Sx. The sample obtained by solvent extraction showed a chemical profile similar to the one obtained by means of SFE but without the added benefit of not having unwanted traces of solvent.

The extracts were evaluated for antioxidant properties, polyphenol content, and inhibitory activity on the xanthine oxidase (XO) enzyme. The antioxidant properties were determined with ABTS assay. The results indicated that the SFE[300:40] extract had low antioxidant activity (EC_{50} = 0.241 ± 0.022 mg/mL) and the Sx extract had no antioxidant activity. The total phenolics of SFE[300:40] extract was 17.7 mg GAE/g of weight. Both extracts showed a very low inhibition activity towards the XO enzyme.

Keywords: *Rosa canina* L.; Supercritical extraction; fatty acids; antioxidant activity; phenolic content.

1. INTRODUCTION

Rosa canina L. (rose hip) is a species of plant belonging to the Rosacea family, which includes about 5000 species. This plant is a shrub up to 3.5 m of height and widespread in northern Europe, Asia, the Middle East, and North America [1,2].

Besides vitamins, minerals, carotenoids, and polyphenols, the rose hip fruit is also a good source of lipid substances as essential fatty acids that humans cannot synthesize and must be take through diet. Essential fatty acids are long-chain polyunsaturated fatty acids derived from linolenic, linoleic, and oleic acids. These chemicals regulate numerous body functions, including blood pressure, blood viscosity, immune, and inflammatory responses [3].

The oil content of rose hip fruits ranges from 5 to 18%. Composed of unsaturated fatty acids such as linoleic acid (36-55%), which is the most abun-

dant one, linolenic (17-27%), and oleic acid (15-22%) respectively [4]. According to the literature, there are few studies on the fatty acid content of rose hip pulp [3]. Data on the chemical composition and biological activity mainly concern seed extracts [5,6]. This work aimed to present a description of the fatty acid profile, the polyphenolic content, and the antioxidant and xanthine oxidase inhibitory activities of extracts derived from the pulp of rosehip fruits obtained using CO_2 in the supercritical state. Xanthine oxidase (XO, EC 1.1.3.22) is an important enzyme that catalyses the oxidation of hypoxanthine to xanthine and subsequently to uric acid. In both steps, molecular oxygen is reduced, forming superoxide anion, followed by the generation of hydrogen peroxide. The overactivity of XO has been associated with the development of gout [7].

Thus, the inhibition of xanthine oxidase can reduce both circulating uric acid levels and the production of reactive oxygen species (ROS) [8]. In this context, the research is directed towards the discovery of extracts with potential beneficial properties, as well as antioxidant, anti-XO, and rich in phenolic compounds, polyphenols and flavonoids.

Supercritical fluid extraction (SFE) is an important alternative to conventional methods. It offers many favourable features over traditional techniques since it uses a clean, inexpensive, non-flammable, and non-toxic solvent. The efficiency of SFE and the bioactive components' extractability are ascribed to many factors such as temperature, pressure, and flow rate [9,10]. In contrast to the organic solvent extraction, SFE works at low temperatures and short process times, thus reducing the thermal damage and degradation of oxygen-sensitive compounds. CO_2 is commonly used as a solvent for SFE, because is non-toxic, inert, non-flammable, odourless, and cheaper [11].

2. EXPERIMENTAL PART

2.1. PLANT MATERIAL

Rosa canina L. fruits were supplied by Minardi (Bagnacavallo-Ravenna, Italy). Before use, the pulp was separated from the seeds and ground using a Malavasi mill (Bologna, Italy).

2.2. SUPERCRITICAL FLUID EXTRACTION

Supercritical CO_2 extraction (SFE-CO_2) was performed in a laboratory apparatus equipped with a 320 cm^3 extraction vessel, as reported by Piras et al. 2017 [12]. Extraction was carried out in a semi-batch mode: batch charging of vegetable matter and continuous flow solvent, adopting an experimental arrangement that leaves out the first separator. About 300 g of *R. canina* pulp was charged in each run. Operative conditions were 300 bar and 40°C in the extraction section and 20 bar and 15°C in the separator. The extract obtained was stored at -20°C *for chemical and biological assays.*

2.3. SOLVENT EXTRACTION IN SOXHLET APPARATUS

Approximately 20 g of material was weighed in a cellulose extraction thimble, which was inserted into the cylindrical part of the apparatus. 60 mL of *n*-hexane was heated to reflux. After 6 h of extraction at a temperature above the solvent boiling point, the solvent was removed from the extract solution using a rotary evaporator until the extract was dried before determining yield. The dry extract obtained was stored at -20°C for chemical and biological assays.

2.4. OIL SAPONIFICATION

The extracts (2 mg, in EtOH solution) obtained from *R. canina* pulp by SFE and Soxhlet extraction were subjected to mild saponification as previously reported [13]. Dried saponifiable fractions, dissolved in acetonitrile with 0.14% acetic acid (v/v), were analysed by high-performance liquid chromatography (HPLC). A portion of dried fatty acid (FA) after saponification was methylated with 3 N methanolic HCl (at room temperature) as reported [14], and FA methyl esters (FAME) were analysed by gas chromatography (GC).

2.5. ANALYSIS OF FATTY ACIDS

FAME were analysed on a gas chromatograph HP-6890 (Hewlett-Packard, Palo Alto, USA) with a flame ionisation detector (GC-FID) and equipped with a cyanopropyl methyl-polysiloxane HP-23 FAME column as reported [13]. FAME were identified with standard compounds and quantified as a percentage of the total amount of FA. After the extract saponification, the analyses of total unsaturated FA (UFA) were carried out with an Agilent Technologies 1100 HPLC system (Palo Alto, CA) equipped with a diode array detector (HPLC-DAD). UFA, detected at a wavelength of 200 nm, were eluted with $CH_3CN/H_2O/CH_3COOH$ (75/25/0.12, v/v/v) as the mobile phase at a flow rate of 2.3 mL/min using an Agilent Technologies XDB-C18 Eclipse column. The chromatogram data were recorded and integrated through an Agilent OpenLAB Chromatography data system. UFA identification was performed using standard compounds and conventional UV spectra. Calibration curves of FA were constructed using standards and were found to be linear (correlation coefficients > 0.995) [13].

2.6. STATISTICAL ANALYSES

Statistical differences were evaluated using Graph Pad INSTAT software (GraphPad Software, San Diego, CA, USA). Student's unpaired t-test assessed comparison between groups with Welch's correction and one-way analysis of variance (One-way ANOVA), followed by the Bonferroni Multiple Comparisons Test. The values with $p < 0.05$ were considered significant.

2.7. DETERMINATION OF TOTAL POLYPHENOL CONTENT (TPC)

The Folin–Ciocalteu test was chosen to measure the TPC of *R. canina* extracts. This test was performed

by referring to the method previously reported [15]. About 5 µL of the extract were mixed with 50 µL of the Folin–Ciocalteu reagent in the test tube. The mixture was allowed to stand for 5 min at room temperature. The mixture was then added about 150 µL of sodium carbonate (Na_2CO_3) aqueous solution, and the test tube was shaken gently to mix them. The absorbance of the mixture was measured using the UV-Vis spectrophotometer at λ = 750 nm.
A calibration curve of standard reference was established using gallic acid (in a range of concentration from 0.01 to 0.1 mM) as standard references plotted. TPC was expressed as gallic acid equivalents, in milligrams per gram of dry weight (dw)

2.8. ANTIOXIDANT ACTIVITY

The method adopted [16] is based on the capacity of an antioxidant to scavenge the free radical $ABTS^{\bullet+}$. The $ABTS^{\bullet+}$ was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate (final concentration) in an aqueous solution, and the mixture was kept in the dark at room temperature for 24 h before use. Extracted samples (10 µL) were added, at different concentrations, to 990 µL of diluted $ABTS^{\bullet+}$ solution and mixed vigorously. After the reaction at room temperature for 1 min, the absorbance at λ = 734 nm (A734) was measured. The decrease in A734 was calculated, and the results were expressed as the extract concentration necessary to give a 50% reduction in the original absorbance (half maximal effective concentration, EC_{50}).

2.9. XANTHINE OXIDASE INHIBITION ASSAY

Xanthine oxidase (XO) activity was determined spectrophotometrically by measuring the formation of uric acid from xanthine, according to the method previously reported [17].
The xanthine solution was prepared by initially dissolving xanthine in a minimal volume of NaOH, adjusting the pH to 7.5. The XO (from bovine milk) solution was prepared by diluting it to a final concentration of 0.5 U/µL in cold 0.1 M phosphate buffer (pH 7.5). The reaction mixture contained: 435 µL of 0.1 M phosphate buffer, 10 µL of plant extract solution, 30 µL of xanthine solution, and 25 µL of XO. The change in absorbance was recorded at 295 nm for 3 min at room temperature. All assays were performed in triplicate. XO activity was expressed as percent inhibition of XO, calculated as [1-(B/A)].100, where A is the change in absorbance of the assay without the plant extract, and B is the change in absorbance of the assay with the plant extract.

3. RESULTS WITH DISCUSSION

This work concerns the extraction using CO_2 in the supercritical state from the pulp of dried fruits of *R. canina* L. The extraction experiment was carried out at a pressure of 300 bar and a temperature of 40°C. After 4 h of extraction, the extract yield amounted to 0.30% by weight of the charged material. The chemical composition of SFE[300:40] was compared to the one of sample Sx, obtained using *n*-hexane at the boiling temperature in a Soxhlet apparatus (yield = 0.86%).

3.1. FATTY ACID COMPOSITION

Quali-quantitative information on FA that composes the extracts from *R. canina* pulp was obtained by HPLC-DAD and GC-FID analyses. The extract obtained by SFE[300:40] showed a high level of linolenic acid, 18:3 *n*-3, (28.37%) of total FA; linoleic acid, 18:2 *n*-6, (26.74%); palmitic acid, 16:0, (18.20%); and oleic acid, 18:1 *n*-9, (15.72%). Followed by low amounts of stearic acid, 18:0, palmitoleic acid, 16:1 *n*-7, lauric acid, 12:0, and myristic acid, 14:0, Table I. The concentrations of the main unsaturated fatty acids, UFA, in the sample were (121.43 ± 3.21) mg/g, (102.16 ± 2.84) mg/g, and (49.95 ± 2.75) mg/g of

Table I - Fatty acids composition (% of total FA)

Fatty acids	SFE[300:40], %	Sx, %
Lauric (12:0)	1.65 ± 0.29^a	1.99 ± 0.04^a
Myristic (14:0)	1.28 ± 0.05^a	1.17 ± 0.09^b
Palmitic (16:0)	18.20 ± 0.11^a	14.15 ± 0.16^b
Palmitoleic (16:1 *n-7)*	2.01 ± 0.30^a	1.70 ± 0.09^a
Stearic (18:0)	3.98 ± 0.30^a	3.52 ± 0.01^b
Oleic (18:1 *n-9)*	15.72 ± 0.01^d	16.62 ± 0.04^c
Linoleic (18:2 *n-6)*	26.74 ± 0.23^d	32.66 ± 0.21^c
Linolenic (18:3 *n-3)*	28.37 ± 0.23^a	24.94 ± 0.30^b
Arachidic (20:0)	0.65 ± 0.06^b	0.54 ± 0.01^b
Gondoic (20:1)	0.10 ± 0.02^b	0.20 ± 0.13^b
ΣSFA	25.77± 0.47^a	21.38 ± 0.03^b
ΣMUFA	17.83 ± 0.29^b	18.52 ± 0.26^a
ΣPUFA	55.10 ± 0.46^d	57.59 ± 0.51^c
ΣUFA	72.93 ± 0.74^d	76.12 ± 0.25^c

SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Extract analysis was performed in triplicate and all data are expressed as mean values ± standard deviations (SD); (n = 3). Mean values in the same row having different letters are significantly different (P < 0.05; One–way ANOVA followed by the Bonferroni Multiple Comparisons Test).

Table II - Fatty acid composition (expressed as mg/g of oil)

Fatty acids	SFE[300:40], mg/g	Sx, mg/g
Oleic (18:1 *n-9)*	49.95 ± 2.75^a	64.68 ± 0.99^a
Linoleic (18:2 *n-6)*	102.16 ± 2.84^b	141.89 ± 4.12^b
Linolenic (18:3 *n-3)*	121.43 ± 3.21^a	127.78 ± 4.15^a

Extract analysis was performed in triplicate and all data are expressed as mean values ± standard deviations (SD); (n = 3). Mean values in the same row having different letters are significantly different (P < 0.05).

extract for 18:3 *n*-3, 18:2 *n*-6, and 18:1 *n*-9, respectively, Table II.

The quality of the sample SFE[300:40], in terms of its chemical composition, was compared with Sx. The extract obtained by solvent extraction showed chemical profiles similar to the ones obtained using SFE-CO_2 (Tables I and II; Figures 1 and 2) but without the additional benefit of not having unwanted traces of solvent. Interestingly, SFE-CO_2 pulp oil was characterised by a high proportion of PUFA and UFA; UFA to SFA ratio value was 2.8 *versus* 3.6 in Sx.

Our results on the FA profiles of *R. canina* pulp extracts align with those previously reported.

In 1997, Illes et al. [18] conducted an extraction from the *R. canina* peel with supercritical CO_2 at 35°C and 250 bar, obtaining similar yield (0.37%) and similar fatty acid profile for linoleic (51.8%), linolenic (23.1%) and oleic (17.8%) acids.

Ercisli, 2007 [3] reported that the major fatty acid in the hexanic extract was linolenic acid, followed by linoleic and palmitic acids (40.5, 16.4, and 16.0%, respectively). Linoleic (39.5-15.9%), linolenic (26.3-16.9%), oleic (14.4-11.8%), and palmitic (6.7%) acids were previously identified as the main components in the oil obtained by petroleum ether extraction, using a Soxhlet apparatus, of fruits of *R. canina* collected in Portugal by Barros et al. 2010, 2011 [19,20]. Also, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were found in similar proportions.

3.2. TOTAL PHENOL CONTENT

To evaluate the total phenolic content in the fruit extracts, the Folin–Ciocâlteu method was used. *R. cani-*

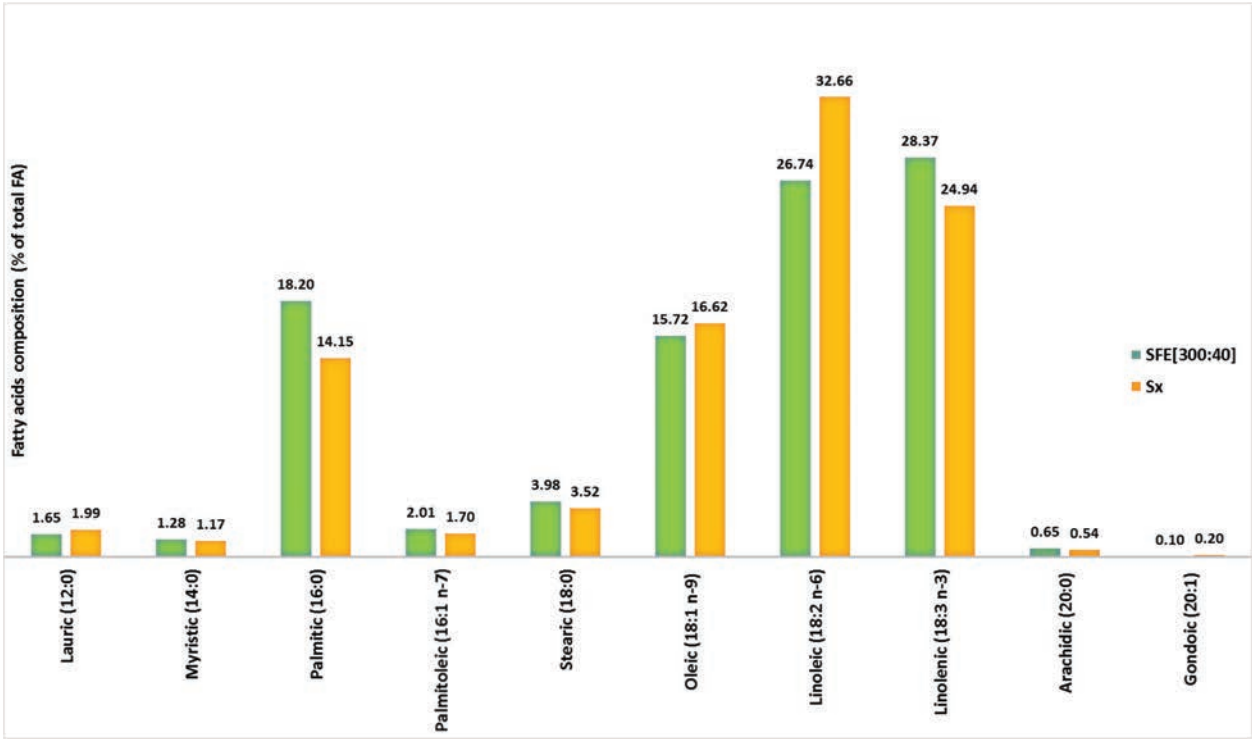

Figure 1 - Fatty acids abundance in *R. canina* extracts.

Figure 2 - Satured and unsatured fatty acids ratio in *R. canina* extracts.

na is a species known for its phenolic compound-rich composition [21]. Our research shows that the total phenolic content, expressed as the concentration of Gallic Acid Equivalents (GAE), in the SFE-CO_2 sample was 17.7 mg/g of extract and a concentration below the detection limit of the method for Sx (Table III). This value is greater than the value found in *R. canina* var. *transitoria* from Romania (5.751 ± 0.1464 mg GAE/g frozen pulp) [2], is lower than the value of 96 mg GAE/g of dry raw fruit from Turkey [3] and of 96.2 ± 4.35 mg GAE/g of extract from Serbia [22].

3.3. ANTIOXIDANT AND XANTHINE OXIDASE INHIBITORY ACTIVITIES

The antioxidant properties were determined by means of the ABTS assay performed in triplicate at different concentrations to estimate the EC_{50} (the concentration of sample required to decrease ABTS radical cation concentration by 50% values). The results (see Table III) indicated that SFE[300:40] possessed antioxidant activity, EC_{50} = (0.24 ± 0.02) mg/mL, and the Sx extract had no antioxidant activity. Both extracts showed a very low inhibition activity towards the XO enzyme. The inhibition percentages were 2.9% for SFE[300:40] and 6.0% for Sx extract.

In conclusion, the main benefit of the SFE method is that it produces a solvent-free, undiluted extract of natural substances applicable for medicinal purposes.

Variations in FAs profiles, polyphenol content, and rose hip pulp's antioxidant activity could result from numerous factors such as climatic, environmental, genetic, etc. No less important, these changes may occur due to the influence of water and enzymes. The aspects of fruit damage degree and humidity of studied raw materials and the drying method, storage conditions, and treatment in the technological process are of great significance for the degree of bioactive compounds.

This study showed the nutritional properties of *R. canina* pulp SFE extract due to their high content of essential fatty acids with health benefits, so qualify these extracts as a potential, environment-friendly, natural resource for food, nutraceutical, and pharmaceutical applications.

Table III - Total phenolic content, antioxidant and inhibitory activity of Xanthine oxidase (I_{XO}).

Extract	Total phenol (mg GAE/g)	ABTS EC_{50} (mg/mL)	I_{XO} (%)
SFE[300:40]	17.7 ± 1.5	0.24 ± 0.02	2.9 ± 2.6
Sx	N.C.	N.A.	6 ± 4
Trolox[a]		0.0013 ± 0.0004*	

N.C.: no classified; N.A.: no activity; Extract analysis was performed in triplicate and all data are expressed as mean values ± standard deviations (SD); (n = 3). GAE = gallic acid equivalent; [a] positive control; *A. Rosa et al. 2017

Disclosure statement

We declare the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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