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CHEMICAL COMPOSITION OF SEED OIL FROM RED GRAPE VARIETIES OBTAINED BY SUPERCritical CO₂ EXTRACTION

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Abstract. This study aimed to investigate the potential of the most abundant red grape variety from the central part of Serbia (the region of three Morava rivers) for grape oil manufacturing, using supercritical CO₂ extraction. The content of the main constituents of grape seed oils from the indigenous variety Prokupac was determined and the results of the analysis were compared with selected, most commonly grown, international varieties (Cabernet Sauvignon, Pinot Noir, Merlot, and Gamay). Fatty acid profile and the content of biologically active compounds (total phenolic compounds and α-tocopherol) were determined. Oxidative stability of the oils was estimated using a DSC (differential scanning calorimetry) method, by determining the oxidation onset temperature. The fatty acid composition was in accordance with literature data. In terms of α-tocopherol content, its low relative amount was measured in the oils from all grape seed varieties.

Key words: grape seed oil, supercritical CO₂ extraction, red grape varieties, oil constituents

1. INTRODUCTION

To obtain satisfying extraction yield and quality of grapeseed oil, many researchers are engaged in the optimisation of isolation methods. Alongside reknown varieties, grown all over the world, certain indigenous varieties, characteristic of the particular region, are also investigated.
Grapeseed oil represents a suitable alternative for traditionally used edible vegetable oils, especially in controlled diets. The health beneficial aspect or functionality of this oil originates from its composition: grape seed oil is rich in unsaturated fatty acids (especially linoleic) and it possesses very low amounts of cholesterol (Bail et al., 2008; Crews et al., 2006; Matthäus, 2008; Pardo et al., 2009).

Grapeseed oil content varies between 7 and 20% (Choi and Lee, 2009; Crews et al., 2006; Dimić and Turkulov, 2000; Luque-Rodríguez et al., 2005; Matthäus, 2008) and depends on grape variety (Luque-Rodríguez et al., 2005), degree of maturity, climate and grapevine cultivation conditions (Pardo et al., 2011). Approximately 90% of the total lipid fraction of the grape seed oil are unsaturated fatty acids, where the most abundant ones are linoleic (58-78%) and oleic acids (3-25%) (Bail et al., 2008; Crews et al., 2006; Luque-Rodríguez et al., 2005; Matthäus, 2008; Pardo et al., 2009; Pardo et al., 2011; Rubio et al., 2009).

Grapeseed oil contains natural antioxidants, such as polyphenols and tocopherols, which have their role in protecting from free radicals (Maier et al., 2009). It is considered that grapeseed oil represents a rich source of α-tocopherol which contributes to the oil stability (Luque-Rodríguez et al., 2005). The content of α-tocopherol in the oil is an important parameter in assessing the biological value of the oil.

However, due to their polarity, the number of polyphenolics that pass into the oil during the extraction process is limited (Joshi et al., 2001; Nakamura et al., 2003). Pardo and co-workers (2009) examined the quality of oils obtained from four red grape varieties grown in two regions in Spain, the Castilla-La Mancha and Murcia regions. The content of total phenolic compounds (TPC) ranged from 10.68 to 34.43 mg/kg. Bail and co-workers (2008) determined that the content of TPC in white varieties varies from 59.0 to 115.5 µg/g.

Supercritical CO₂ extraction is an environmentally acceptable method of oil isolation. Liquid CO₂ is a non-toxic non-flammable, cheap, and recyclable solvent that can be easily eliminated from the extract at atmospheric pressure, by extract depressurization. Another fact is that critical conditions are easily achieved (Beveridge et al., 2005; Cao and Ito, 2003; Gomez et al., 1996). Fluids in a supercritical state possess higher solubilizing properties and it implies higher process selectivity under classical solvent extraction conditions. The physical and chemical properties of supercritical fluids can vary significantly without change at the molecular level (Sovilj, 2004). A minor variation of pressure and/or temperature causes a change in constituent solubility. A supercritical fluid has a higher diffusion coefficient, even 100 times higher than common solvents, lower viscosity, and surface tension, which leads to a more favorable mass transfer (Abbas et al., 2008; Sovilj, 2004).

This study aimed to investigate the potential of the most abundant red grape variety from the central part of Serbia (the region of three Morava rivers) for grape oil manufacturing, using supercritical CO₂ extraction. The content of the main constituents of grape seed oils from the indigenous variety Prokupac was determined and the results of the analysis were compared with selected, most commonly grown, international varieties (Cabernet Sauvignon, Pinot Noir, Merlot, and Gamay). Fatty acid profile and the content of biologically active compounds (total phenolic compounds and α-tocopherol) were determined. Oxidative stability of the oils was estimated using a DSC (differential scanning calorimetry) method, by determining the oxidation onset temperature.
2. MATERIALS AND METHODS

2.1. General

Seeds of four international varieties of red grape (Cabernet Sauvignon (C. S.), Merlot (M), Pinot Noir (P. N.), Gamay (G)) and indigenous variety Prokupac, cultivated in the vineyard of wine producer “Rubin” Kruševac in Serbia harvested in 2013, were used in this study. Seeds were separated during the fermentation process using a special valve on the vessel. Clean seeds were dried on the ambient air to a moisture content below 10%. Dry seeds were ground with a horizontal coffee grinder (Elektron, Dublje) and sifted through standard laboratory sieves with 0.3 mm and 0.5 mm pore size. The fraction with an average particle diameter of 0.4 mm collected between sieves was used for the experiments.

Carbon dioxide (99.9%) and synthetic air were purchased from Messer Technogas A.D. (Novi Sad, Serbia). n-Hexane, methanol, ethanol, boron trifluoride, sodium hydroxide, potassium hydroxide, sodium carbonate, sodium thiosulphate, hydrogen peroxide, hydrogen chloride were purchased from Merck KgaA (Darmstadt, Germany). Gallic acid was purchased from Alfa Aesar-Thermo Fisher GmbH (Kandel, Germany) and α-tocopherol was purchased from Acras Organics-Thermo Fisher GmbH (Germany).

Laboratory-scale equipment (Autoclave Engineers SCE Screening Systems, USA) was used for the extraction of grapeseed oils. Oil isolation was performed with 25.0 g of seed samples, at the temperature of 50 °C, the pressure of 25.0 MPa, and a flow rate of 0.3 kg CO₂/h.

2.2. Total Oil Content - Soxhlet Method

Soxhlet method was used to determine total oil content according to standard method SRPS EN ISO:2011. Extraction was conducted at 80 °C for 6 h. The solvent was removed at rotary vacuum evaporator „Senco“, at 50 °C, 0.8 bar and 150 r/min.

2.3. Oil Yield

Oil yield was calculated by measuring the mass of the seed samples before extraction (mseed) and the mass of the obtained oil (moil). The following equation was used:

\[ Y(\%) = \frac{m_{oil}}{m_{seed}} \times 100 \]  

(1)

2.4. Fatty Acids Composition

The standard gas chromatographic method was applied to analyze the fatty acid composition of the oils (IUPAC, 1992). Transesterification of triglycerides extracted from oils was done using a 12% solution of BF₃ in methanol (v/v) (IUPAC, 1992). Quantitative GC analysis was performed on a GC5160 gas chromatograph (Carlo Erba, Milano, Italy), capillary column (30 m × 0.25 mm ID × 0.25 μm film) ZB-5 ms (Phenomenex, USA). The chromatographic conditions were as follows: 1 μL splitless injection at 100 °C, 3 min isothermal time, a ramp of 10 °C/min to 220 °C, 20 min isothermal time, and the second ramp of 40 °C/min to 300 °C. The injector temperature
was 270 °C and the splitless time was 60 s. The flow rate of the carrier gas (helium) was 1.5 mL/min and the detector (flame ionization detector) temperature was set at 300 °C. The internal standard method was used for fatty acids quantification. A calibration curve was constructed using pentadecanoic acid as an internal standard.

2.5. Total Phenolic Compounds (TPC)

Quantification of the total phenolic content of the investigated grape seed oils was carried out using the colorimetric method described in the literature (Pardo et al., 2009; Ceci and Carelli, 2010), based on the reaction of the Folin-Ciocalteu reagent with hydroxyl groups. Phenolic compounds were extracted by diluted methanol (90:10), for 5 minutes, on an ultrasound bath. The extracts were centrifuged at 4000 rpm, for 10 minutes. The extractions were done in triplicate. All the methanolic extracts were combined and concentrated until dryness. The dry residue was dissolved with 2.5 ml diluted methanol (90:10) and kept at -20 °C before analysis. For spectrophotometric determination, 0.5 mL of the oil sample was mixed with Folin-Ciocalteu reagent according to the procedure described in the literature (Bail et al., 2008; Faller and Fialho, 2010). The absorbance was recorded at 730 nm. Quantification was carried out using a standard curve, and results were expressed as milligrams of gallic acid equivalents per 100 g of oil (mg GAE/100g).

2.6. α–Tocopherol Content

HPLC method was used to determine the α-tocopherol content in the oil, according to the procedure published by Dabbou and co-workers (2010). The oil sample (0.0100-0.0200 g) was dissolved in 1 mL of 2-propanol. The analysis was performed at room temperature on a Bischoff (Bischoff, Leonberg, Germany) HPLC chromatograph equipped with a Rheodyne 7725i sample injector with a loop of 20 μL, a compact pump model 2250, with a UV/VIS Lambda 1010 as the detection system (Bischoff, Germany), an in-line degasser (Bischoff, DG 1410) and with a Control and Data Interface LC-CaDI 22-14 (with McDaqc32-Control software Bischoff). α-tocopherol was separated on a C18 reversed-phase column (250 mm × 4.6 mm, 120A, 5 μm, ProntoSil, Bischoff). Isocratic elution with methanol:water (90:10%, v/v) at a flow of 1 mL/min was applied. α-tocopherol was monitored at 280 nm, at 19.25 min retention time.

2.7. Oxidative Stability

Oxidative stability of the oils was examined by a procedure defined by ASTM standard E2009-08, and already used for that purpose (Ulkowski et al., 2005). The onset point of the exothermic DSC (differential scanning calorimetry) signal which occurred during the controlled heating of the oil sample, was used for the assessment of the oxidative stability. Measurements were performed on DSC/TG apparatus (DSC/TG111, Setaram, France), under atmospheric pressure. The Setsoft software (Setaram, France) was used to collect data and for the determination of temperatures from DSC curves. The apparatus was calibrated with high-purity indium. The experiments were performed under a flow of synthetic air (22% of O₂ and 78% of N₂) at a flow rate 25 dm³/min. Samples of oils were heated from 20 to 200 °C in an open quartz crucible at a linear heating rate of β = 10 °/min; an empty quartz crucible was used as reference material.
2.8. Statistical Analysis

All experimental experiments were performed in triplicate. Statistical Package for Social Sciences (IBM SPSS Statistics 20, Chicago, IL, USA) was used for performing statistical analysis. The differences between the mean values were evaluated by a Tukey test with significant levels set at \( p \leq 0.05 \). Results given as mean values ± SD (standard deviation) were further subjected to one-way analysis of variance (ANOVA). For those parameters for which the significance of mean value differences was shown, the Duncan test for post hoc analysis was used.

3. RESULT AND DISCUSSION

Chemical analysis of the oils extracted from the grape seeds of different grape varieties included the analysis of fatty acids, total polyphenolic compounds, \( \alpha \)-tocopherol, and the determination of oxidative stability.

3.1. Fatty Acids Composition

The composition and the content of the unsaturated fatty acids have the main influence on the oxidative processes in plant oils (Min and Boff, 2002; Reische et al., 2002). Kamal-Edin (2006) reported that oxidative stability significantly depends on the degree of unsaturation. Quantification of the fatty acids in the oils obtained from different varieties of the grape was done by the GC method and the results are presented in Table 1.

GC analysis revealed that the oils contain saturated (palmitic, stearic, arachidic, and behenic) and unsaturated (oleic, linoleic, and linolenic) fatty acids. Unsaturated fatty acids ranged from 87.59 to 89.07%. The highest content of unsaturated fatty acids was found in the oil obtained by the extraction of the seed of the grape variety Gamay (89.07%), while the lowest content was found in the oil isolated from the grape seeds of the variety Cabernet Sauvignon (87.59%). The prevalent fatty acid in all analyzed oils was linoleic acid (C18:2, \( \omega-6 \)), found in the range 70.89-73.99%. There was a statistically significant difference among oils extracted from different grape varieties (\( p<0.05 \)). The oil from the grape variety Merlot was marked as the oil with the highest content of linoleic acid and the lowest content of this acid was found in the oil obtained from the grape variety Pinot Noir. The second most abundant fatty acid was oleic acid with the content ranging from 14.06% (oil from the Merlot variety) to 17.25% (oil from the Gamay variety). Linolenic acid (C18:3, \( \omega-3 \)) was found as a minor unsaturated fatty acid (0.35 - 0.42%) in all analyzed oils. Palmitic acid was detected as the major saturated fatty acid (7.33-7.99%), followed by stearic acid (3.31-4.10%). Behenic and arachidic acids were minor oil components in all investigated oils.

Statistical analysis showed that the fatty acids composition was significantly influenced by grape variety (\( p<0.05 \)), which is marked by a capital letter A-D. It is especially evident in the content of the most dominant acids (linoleic and oleic).
Table 1 The fatty acid content (%) of the grapeseed oils obtained by supercritical CO2 extraction of different grape varieties

| Fatty acid (%) | Grape Variety | Pinot Noir (P.N.) | Gamay (G) | Prokupac (P) | Cabernet Sauvignon (C.S.) | Merlot (M) | Average |
|---------------|---------------|------------------|-----------|-------------|--------------------------|-----------|---------|
| C-16:0        |               | 7.73±0.03        | 7.33±0.04 | 7.87±0.03   | 7.99±0.01                | 7.65±0.01 | 7.72    |
| C-18:0        |               | 3.53±0.014       | 3.31±0.003| 3.59±0.002  | 4.10±0.005               | 3.66±0.006| 3.64    |
| C-18:1        |               | 17.19±0.023      | 17.25±0.011| 16.25±0.008 | 14.3±0.023               | 14.06±0.005| 15.81  |
| C-18:2        |               | 70.89±0.003      | 71.47±0.014| 71.61±0.032 | 72.9±0.074               | 73.90±0.005| 72.17  |
| C-18:3        |               | 0.36±0.006       | 0.35±0.013 | 0.42±0.008  | 0.39±0.018               | 0.37±0.002| 0.38    |
| C-20:0        |               | 0.12±0.001       | 0.12±0.004 | 0.14±0.004  | 0.16±0.006               | 0.15±0.002| 0.14    |
| C-22:0        |               | 0.18±0.006       | 0.17±0.020 | 0.13±0.002  | 0.16±0.006               | 0.12±0.011| 0.15    |
| SFA           |               | 11.56            | 10.93     | 11.73       | 12.41                    | 11.48     | 11.64   |
| MUFA          |               | 17.19            | 17.25     | 16.25       | 14.30                    | 14.06     | 15.81   |
| PUFA          |               | 71.25            | 71.82     | 72.02       | 73.29                    | 74.21     | 72.55   |
| UFA           |               | 88.44            | 89.07     | 88.27       | 87.59                    | 88.42     | 88.36   |

Data are expressed as a mean value ± SD (n=3). Means with a different letter in a column are statistically significant at 5% level probability by Tukey Test (p<0.05)

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acid, UFA – total unsaturated fatty acids

It was found that oils of the tested varieties are a good source of polyunsaturated fatty acids (PUFA) (71.25%-74.21%). By examining ten traditional Portuguese grape varieties with a satisfactory chemical and antioxidant profile, Fernandes and co-workers (2013) found slightly lower content of the PUFA (63.64%-73.53%).

Using supercritical fluid extraction with ultrasound as a pretreatment de Souza and co-workers (2020) extracted oil with a lower level of the MUFA (5.68%) and PUFA (62.82%) and a significantly higher level of saturated fatty acids (31.51%). Experiments were carried out at the same temperature (50 °C), higher pressure (50 MPa), and a similar CO2 flow of 6 g/min (0.36 g/min in our experiments).

Red grape varieties (a mixture of Cabernet Sauvignon, Merlot, and Pinot Noir) were the samples for the examination of the modern technique of oil isolation and similar fatty acids were identified (Dimić et al., 2020). Large variation between the varieties and year of harvest (area of South Brazil) was found in the grape seed oils isolated from Merlot and Cabernet Sauvignon. The supercritical CO2 extraction method was applied at the same pressure and higher temperature and CO2 flow rate compared to our experimental conditions (80 °C, 4.14 kg CO2/h) (Agostini et al., 2012).

Beveridge and co-workers (2005) used the same international varieties for supercritical CO2 extraction. Similar results were found for some fatty acids and varieties (content of palmitic and stearic acid for Merlot and Gamay varieties). Results also showed that oils obtained from the seeds of the Merlot variety were the richest in linoleic acid. Those differences could be attributed to different extraction parameters, growing, and climate conditions. It is important to say that in all samples oleic acid was found in a higher amount than it was previously reported (13.13-14.74%) (Beveridge et al., 2005).

Other authors that used supercritical CO2 extraction reported similar composition of the lipid fraction of grapeseed oils (Pérez et al., 2015, Ramazanov et al., 2018). Behenic acid, which was detected in all investigated oils, was not reported by the aforementioned
Chemical Composition of Grape Seed Oil Obtained by Supercritical CO$_2$ Extraction

Supercritical CO$_2$ extraction is acceptable as a method for oil isolation from the point of fatty acids composition.

3.2. Content of $\alpha$-Tocopherol and Total Phenolic Compounds

Mono- and polyphenols and $\alpha$-tocopherol act as primary antioxidants (Reische et al. 2002). $\alpha$-Tocopherols are considered as highly sensitive compounds to light and air (oxygen) and because of that, they lose antioxidant potential during the sample preparation (Beveridge et al. 2005, Freitas et al., 2008). The content of $\alpha$-tocopherol in the grapeseed oils obtained from red grape varieties by supercritical CO$_2$ extraction is shown in Fig. 1.

![Graph](image)

**Fig. 1** Total phenolic compounds content (TPC), g/100 g (a), and $\alpha$-tocopherol content, mg/100 g (b) in the grape seed oils obtained from red grape varieties by supercritical CO$_2$ extraction (P. N. – Pinot Noir, G – Gamay, P – Prokupac, C. S. – Cabernet Sauvignon, M – Merlot)

As we can see, the obtained values lay in a very narrow range (0.100 mg/100 g - 0.308 mg/100 g) with an average value of 0.27 mg/100 g. Besides the narrow range, there is a statistically significant difference between these values. Grapeseed oil obtained from the variety Prokupac showed the highest $\alpha$-tocopherol content, while the lowest one was found in variety Gamay. Beveridge (2005) and Bravi (2007) with their co-workers found a higher $\alpha$-tocopherol level (70.67 and 30.9 mg/100 g respectively). Those authors used different extraction parameters compared to our study (temperature of 65 ºC and 370 bar pressure - Beveridge et al. (2005), the temperature of 80 ºC and 250 bar pressure - Bravi et al. (2005)). For the grape varieties Merlot and Cabernet Sauvignon grown in Brazil (Agostini et al., 2012), quality examination of the extracted oils, obtained by supercritical CO$_2$ extraction, was shown that the $\alpha$-tocopherol level was close to our findings (0.17-0.37 mg/100 g - Merlot, and 0.14-0.26 mg/100 g - Cabernet Sauvignon). They applied pressure of 250 bar and 80 ºC. The quantity of the $\alpha$-tocopherol in the grape seed oils was highly influenced by extraction parameters in supercritical CO$_2$ extraction, as well as a grape variety. The content of total phenolic compounds in obtained oil extracts is presented in Fig. 1.

Content of the total phenolic compounds showed variation between varieties, ranging from 4.6 mg/100 g to 7.88 mg/100 g. It was found that there was no statistically significant
difference in oils from grape variety Merlot and Gamay. For other oils, a significant difference was determined. Results led to the conclusion that the level of the extracted phenolic compounds was variety-dependent. At given extraction conditions, the highest content of total phenolic compounds was found in oil from the Prokupac variety. Content of total phenolic compounds was higher than those reported by Agostini et al. (2012); they found that the TPC content in the Merlot variety was from 1.57 to 1.74 mg/100g, and from 2.06 to 2.13 mg/100g for the Cabernet Sauvignon variety. They used the pressure of 250 bar and the temperature was 80 °C. Therefore, it could be concluded that high temperature did not favor the extraction of phenolic compounds. It was observed that the content of these compounds tended to increase with pressure (Pérez et al., 2015). Perez and co-workers observed the lowest TPC value at 200 bar and 60 °C, while the highest TPC was obtained at 300 bar and 40 °C (11.348 mg/100 g). The same was confirmed by de Souza et al. (2020), who investigated grape seed oil extraction methods with and without ultrasound pretreatment. Supercritical extraction from indigenous variety Syrah was conducted at 50 °C and pressure of 500 bar. The content of total phenolic compounds was from 136 to 349 mg/100 g.

3.3. Determination of the Oxidative Stability by DSC Method

DSC method was used to investigate the oils’ resistance to oxidation. Experiments were set in the linear heating regime in the synthetic air. In the non-isothermal conditions, a sharp rise of the exothermic signal and oxidation onset temperature (OOT) was determined and it was taken as a relative measure of oxidative stability. Obtained OOT values are given in Fig. 2.

![Fig. 2 Oxidation onset temperature (OOT) of the grape seed oils from different red grape varieties obtained by supercritical CO2 extraction (g/100g)](image)

Grapeseed oil from the Prokupac variety was recognized as the oil with the highest oxidative stability according to determined oxidation onset temperature (170.33 °C). Grape seed oils extracted from other varieties showed significantly lower values of OOT, ranging from 161.0 to 162.4 °C. A significant difference was not found among those varieties.
There was no correlation between the content of dominant fatty acids (linoleic and oleic acid) and OOT (R²=0.25 for linoleic acid, R²=0.32 for oleic acid). The oils isolated from the grape variety Pinot Noir, Gamay, and Prokupac had a higher content of the monounsaturated oleic acid (C-18:1) and lower content of the polyunsaturated linoleic acid (C-18:2) compared to the oils from grape varieties Cabernet Sauvignon and Merlot, which categorized them as oils less susceptible to oxidation.

Contrary to the fatty acid profile, a good correlation was found between OOT and TPC content (R²=0.88). Oil from varieties Prokupac and Cabernet Sauvignon contained higher levels of the TPC compared to grape varieties Gamay, Merlot, and Pinot Noir, and those two oils had a higher resistance to oxidation processes. Due to the very low content of α-tocopherol in all samples, its influence on oxidation stability should be insignificant. A very low correlation between α-tocopherol level and values for OOT was determined (R²=0.19). It can be concluded that all biologically active compounds of grapeseed oils contributed to oxidative stability, where total phenolic compounds have a very significant role.

4. CONCLUSION

The main constituents of the grapeseed oils obtained by supercritical CO₂ extraction showed a significant variation between varieties. Compared to other investigated varieties, seeds from the indigenous variety Prokupac contained a higher level of α-tocopherol and total phenolic compounds and had a high value of OOT, which make the seeds a good source of biologically valuable oil with good oxidation resistance. A more complete isolation of α-tocopherol and phenolic compounds requires optimization of the extraction parameters.

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**HEMIJSKI SASTAV ULJA SEMENKI CRVENIH SORTI GROŽDA IZOLOVANIH NATKRITIČNOM CO2 EKSTRAKCIJOM**

**Cilj ovog rada** je ispitivanje potencijala za dobijanje ulja iz semenki grožđa najzastupljenijih crvenih sorti grožda u centralnom delu Republike Srbije (oblast tri Morave), primenom natkritične CO2 ekstrakcije. U radu je izvršena karakterizacija glavnih sastojaka semenki autohtone sorte Prokupac i poređenje sastava ovog ulja sa uljima izolovanih iz semenki najzastupljenijih internacionalnih sorti grožđa u ovom regionu. Određen je masnokiselinski sastav ulja, sadržaj ukupnih fenolnih jedinjenja i sadržaj α-tokoferola. Za određivanje oksidativne stabilnosti ulja upotrebljena je DSC metoda. Sastav masnih kiselina u uljima bio je u skladu sa podacima iz literature. Utvrđeno je da se od svih sorti grožđa primenjenim uslovima ekstrakcije dobijaju ulja sa niskim sadržajem α-tokoferola.

**Ključne reči:** Ulje iz semenki grožđa, natkritična CO2 ekstrakcija, crvene sorte grožđa, sastav ulja