

Characterization of fatty acid, antioxidant, and polyphenol content of grape seed oil from different *Vitis vinifera* L. varieties[☆]

Viktória Kapcsándi, Erika Hanczné Lakatos, Beatrix Sik, László Ádám Linka and Rita Székelyhidi^{*}

Department of Food Science, Faculty of Agricultural and Food Sciences, Széchenyi István University, Lucsony street 15-17, 9200 Mosonmagyaróvár, Hungary

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Abstract – In this study, we examined the yield and oil quality of eight different grape varieties. For the experiments, the grape seeds were obtained from the Benedictine Pannonhalma Archabbey in the northwestern region of Hungary. The aim of the studies was to determine the oil yield obtained by extraction and to examine the differences between the fatty acid composition, antioxidant capacity, and total polyphenol content of the oils of different grape varieties. Based on the results, the oil content of the grape seeds varied between 99.91 g/kg and 126.74 g/kg. The grape seed oils analysed contained significant amounts of stearic acid (3.42–9.93%), palmitic acid (7.81–10.66%), oleic acid (14.29–19.92%), and linoleic acid (66.85–72.47%). Besides, the grape seed oils tested contained several other fatty acids in small amounts. There were significant differences in the total antioxidant and total polyphenol content of the oils. Total polyphenol content ranged from 0.24 to 1.13 mg GAE/g, while the total antioxidant content changed between 0.12 and 0.78 μ g TEAC/g. The results show that the studied varieties are suitable for the production of table grape seed oil based on their oil yield, and the oils have favourable, health-protecting properties in terms of their quality.

Keywords: grape seed oil / oil yield / fatty acid composition / polyphenols / antioxidants

Résumé – Caractérisation de la teneur en acides gras, en antioxydants et en polyphénols de l'huile de pépins de raisin de différentes variétés de *Vitis vinifera* L.. Dans cette étude, nous avons examiné le rendement et la qualité de l'huile de huit différentes variétés de raisin. Pour les expériences, les pépins de raisin ont été obtenus de l'abbaye bénédictine de Pannonhalma dans le Nord-Ouest de la Hongrie. L'objectif était de déterminer le rendement en huile obtenu par extraction et d'examiner les différences de composition en acides gras, capacité antioxydante et teneur totale en polyphénols des huiles issues de différents cépages. D'après les résultats, la teneur en huile des pépins de raisin variait entre 99,91 g/kg et 126,74 g/kg. Les huiles de pépins de raisin analysées contenaient des quantités significatives d'acide stéarique (3,42–9,93 %), d'acide palmitique (7,81–10,66 %), d'acide oléique (14,29–19,92 %) et d'acide linoléique (66,85–72,47 %). En outre, les huiles de pépins de raisin testées contenaient plusieurs autres acides gras en petites quantités. Des différences significatives dans la teneur en antioxydants totaux et en polyphénols totaux des huiles ont été notées : la teneur en polyphénols totaux variait de 0,24 à 1,13 mg GAE/g, tandis que la teneur en antioxydants totaux variait de 0,12 à 0,78 μ g TEAC/g. Les résultats montrent que les variétés étudiées conviennent à la production d'huile de table issue de pépins de raisin au regard de leur rendement en huile, et que, sur le plan qualitatif, les huiles possèdent des propriétés bénéfiques et protectrices sur la santé.

Mots clés : huile de pépins de raisin / rendement en huile / composition en acides gras / polyphénols / antioxydants

[☆] Contribution to the Topical Issue “Minor oils from atypical plant sources / Huiles mineures de sources végétales atypiques”.

^{*}Correspondence: szekelyhidi.rita@sze.hu

1 Introduction

Grape (*Vitis vinifera* L.) is one of the most widely grown fruit in the world. Nearly 80 million tons of grapes are harvested annually (OIV, 2019). Much of this is consumed in the form of fresh fruit, juice or raisins. The other part of the harvested grapes is used for winemaking, it also shows that wine is one of the most popular alcoholic beverages (Kallithraka *et al.*, 2006; Teixeira *et al.*, 2014). The most important wine regions are in Europe (Italy, Spain, France, Germany and Portugal), North and South America (United States, Argentina, Chile) Asia (China) (OIV, 2019), Australia and South Africa. However, the cultivation and processing of grapes also involves the production of large quantities of by-products such as grape marc, grape seed, grape skin, grape stem and grape leaf. Most of these by-products contain large amounts of physiologically important phenolic compounds. Grape marc is the main by-product that remains after pressing; which is about 20% of all grapes processed in wineries (Goula *et al.*, 2016; Bordiga *et al.*, 2019). Grape marc is a basic by-product of wine production, which also includes 20–26% grape seed with the valuable oil and proteins it contains (Mattos *et al.*, 2017). Thirty-five thousand tonnes of grape seed oil are produced worldwide each year, of which 10 000 tonnes come from French factories (Pierron, 2017).

The grapes should ideally contain 4 seeds, which are formed from the four seed buds in the seed coat. In exceptional cases, more seeds may be formed, but usually less than four seeds are typical, 13% of the total weight of grapes is grape seed. The complete absence of seeds can be a characteristic of the grape variety, this is an ideal property in the case of table grapes and raisins. The nutrition of grape seed is physiologically important as it contains roughly 32–43 m/m% dietary fiber, 7–17 m/m% protein and about 5–8 m/m% complex phenolic compound, as well as sugars, mineral salts, etc. (Fantozzi, 1981; Shi *et al.*, 2003; Campos *et al.*, 2008; Tangolar *et al.*, 2009; Mattos *et al.*, 2017).

In addition, grape seeds contain 8–20% oil, which is rich in essential fatty acids. The amount of oil that can be extracted depends on the grape variety and the extraction process used (Bail *et al.*, 2008). The chemical composition of the extracted oil is influenced by the degree of ripeness and species of the seeds, the environmental parameters, the cultivation works and to a small extent the seed extraction protocol (Shinagawa *et al.*, 2015; Garavaglia *et al.*, 2016; Martin *et al.*, 2020). Despite these factors, unlike grapes, grape seeds and their extracts, especially grape seed oil, contain large amounts of lipophilic molecules, among which are several important biocomponents.

The most common of these molecules are fatty acids. In general, about 90% of the total amount of grapeseed oil is mono- and polysaturated fatty acids. It contains in the highest amount of linoleic acid (58–78%), oleic acid (3–15%), and to a lesser extent other saturated fatty acids (10%) (Bail *et al.*, 2008; Rombaut *et al.*, 2015; Konuskan *et al.*, 2019).

The second largest group of lipophilic molecules in grape seeds are vitamins. Phytosterols are also important lipophilic molecules in grape seed oil, but their amount is influenced by harvest conditions and oil extraction methods. The biological importance of phytosterols lies in their antioxidant activity and

their role in cholesterol metabolism (Shinagawa *et al.*, 2015; Garavaglia *et al.*, 2016).

Phenols are natural molecules with antioxidant effects and are present in grapes, especially in grape seeds and their extracts. However, hydrophilic phenols make up only a small proportion of grape seed oil (Fernandes *et al.*, 2013; Khurana *et al.*, 2013), but properly selected oil extraction processes can increase the phenol content of oils (Maier *et al.*, 2009; Rombaut *et al.*, 2015). Among the phenolic compounds, grape seed oil contains mainly gallic acid, catechin, epicatechin, procyanidins and proanthocyanidins or condensed tannins, which are known for their antioxidant activity (Garavaglia *et al.*, 2016).

In this study, grape seed oil was produced from eight different grape varieties using Soxhlet extraction, which was grown in the northwestern region of Hungary by the Benedictine Archabbey of Pannonhalma. The aim of the studies was to determine the oil yield obtained by solvent extraction and to investigate the differences between the fatty acid composition, antioxidant capacity and total polyphenol content of the oils of different grape varieties.

2 Materials and methods

2.1 Grape seed samples

The dried grape seed samples with a moisture content of 7–8% (on a dry matter basis) were obtained from the Benedictine Pannonhalma Archabbey (Hungary). The studied varieties were the following: “Italian Riesling”, “Cabernet Franc”, “Pinot Noir”, “Sauvignon Blanc”, “Királyleányka”, “Rhine Riesling”, “Merlot”, and “Lemberger”. The dried samples were chopped using a coffee grinder (Sencor, SCG 2050RD) and stored away from light until analysis.

2.2 Chemicals

Petroleum ether (Carlo Erba, Spain) boiling at 40–70 °C was used for Soxhlet extraction. Sodium hydroxide, sodium chloride, n-hexane, boron-trifluoride in methanol were purchased from Merck (Germany) to determine fatty acid composition. Supelco 37 FAME Mix (Sigma-Aldrich, USA) was used to identify fatty acids. Chemicals for the determination of polyphenol and antioxidant content were 97% ethanol (Reanal, Hungary), anhydrous sodium carbonate (Riedel-de Haën, Germany), Folin-Ciocalteu reagent (Merck, Germany), 2-4-6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich, Hungary), acetic acid (Reanal, Hungary), anhydrous iron chloride (Merck, Germany), 98% Trolox (Sigma-Aldrich, Hungary), gallic acid (Sigma-Aldrich, Hungary). Nitrogen (4.6) and helium (5.0) was purchased from Linde (Hungary).

2.3 Oil extraction from grape seeds

2.3.1 Soxhlet-extraction

The milled grape seeds (10 g) were extracted with 170 mL petroleum ether for 3 h at a maximum temperature of 70 °C in a Soxhlet apparatus. After extraction was completed, petroleum ether was evaporated by a rotary evaporator (Bibby, RE 100).

The oils obtained were weighed and the yields were calculated. Grape seed oils were weighed into 4 mL screw cap vials and stored in an ultra-freezer at -55°C for further analysis.

2.4 GC-MS method

2.4.1 Sample preparation

Six mL of 0.5 M NaOH was added into a round bottom flask containing 25 mg grape seed oil. Samples were extracted for 6 min at 70°C using a laboratory water bath equipment. After the oil droplets had dissolved, the solution was heated with 70 mL of boron trifluoride in methanol (14%) for 2.5 minutes at 70°C . Finally, 4 mL n-hexane and 6 mL saturated NaCl solution were added and vortexed. For analysis, the methyl-esterified sample was taken from the upper hexane phase, which was first removed and concentrated under nitrogen.

2.4.2 GC-MS analysis

For the determination of fatty acid composition of the oils a Shimadzu (Kyoto, Japan) GCMS-QP2010 SE type equipment was used. Fatty acids were separated on a Zebtron BPX-70 ($30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$) column (Phenomenex, USA). The applied temperature program was 60°C – 120°C with a heating rate of $13^{\circ}\text{C}/\text{min}$, then 120°C – 240°C with a heating rate of $2^{\circ}\text{C}/\text{min}$, and finally 240°C for 8 minutes. One μL of sample solutions was transferred to the 220°C injector of the GC-MS in split mode (split ratio 40). Helium was used as carrier gas with a linear flow rate ($1\text{ mL}/\text{min}$).

2.5 Determination of total antioxidant and polyphenol content

2.5.1 Sample preparation

To determine the antioxidant and polyphenol content, grape seed oils were extracted by solvent extraction technique as follows. Briefly, a mass of 1 g of oil was extracted with 5 mL of ethanol/water (70:30 v/v%) at 60°C for 5 min by means of an RF-120F ultrasonic bath (Realsonic, Hungary). The oily extracts were filtered through a filter paper, and the filtrate was used for further analysis.

2.5.2 FRAP assay

Two hundred μL of extracted sample, 3 mL of FRAP solution, and 100 μL of water were pipetted into a test tube. The finished solutions were placed in a dark place for 5 min and then their absorbance was measured with a Spectroquant Pharo 100 spectrophotometer (Merck, Germany) at a wavelength of 593 nm against the blank. Trolox was used as a standard ($1\text{--}30\text{ }\mu\text{g}/\text{mL}$) and the results were expressed as μg Trolox equivalent antioxidant capacity (TEAC)/g oil.

2.5.3 Folin–Ciocalteu assay

To 200 μL of grape seed oil extract, 1.5 mL of high purity water was pipetted and the reagents were added. First 2.5 mL of 10% Folin–Ciocalteu reagent, then 2 mL of 7.5% Na_2CO_3 . The test tubes containing the mixture were placed in a dark place for 90 min, and then the absorbance was measured at

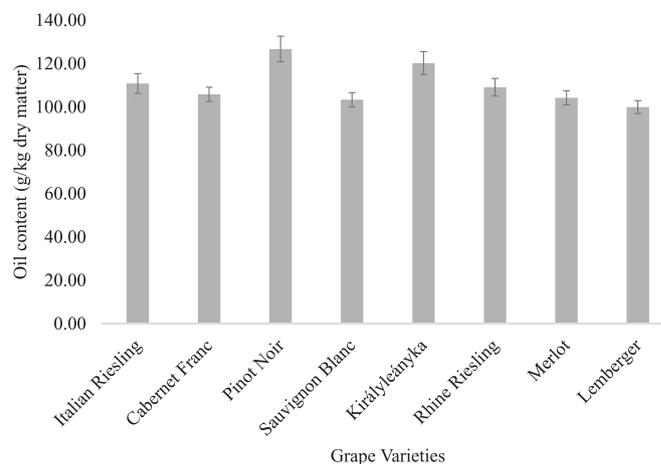


Fig. 1. Oil yield of grape seeds of different cultivars (g/kg).

725 nm versus the blank. Gallic acid was used as a standard ($25\text{--}100\text{ mg}/\text{mL}$).

2.5.4 Data analysis

The total antioxidant and polyphenol contents of grape seed oils were determined in Microsoft Office Excel from the absorbance values measured for grape seed oils using the equation of the second-order least squares analytical curve fitted to the measurement solutions using the nonlinear least-squares method. All the results are expressed as means ($n = 3$) \pm standard deviation.

3 Results and discussion

3.1 Oil yield

During the Soxhlet extraction, the oil content of each grape seed sample was determined gravimetrically. During the evaluation, we present our results in terms of g/kg dry matter (Fig. 1).

As shown in Figure 1, the grape seed cultivars we studied provided similar results in oil yield. Significant differences were found, the difference between the highest and lowest oil yield was $26.83\text{ g}/\text{kg}$ dry matter. The highest amount of oil was extracted from “Pinot Noir” ($126.74\text{ g}/\text{kg}$) and the lowest amount from “Lemberger” ($99.91\text{ g}/\text{kg}$) grape seed meal.

Among grape varieties, seeds of Sauvignon Blanc showed the lowest percentage yield (10.33%), while the oil yield from “Pinot Noir” was the highest (12.67%). “Királyleányka” contained the second-highest yield of oil (12.02%), followed by “Italian Riesling” (11.08%), Rhine Riesling (10.91%), “Cabernet Franc” (10.58%), and “Merlot” (10.47%). These oil yields correspond to the 8–20% oil content reported in the literature (Crews *et al.*, 2006; Bail *et al.*, 2008; Tangolar *et al.*, 2009; Sabir *et al.*, 2012; Elagamey *et al.*, 2013; Fernandes *et al.*, 2013; Lachman *et al.*, 2015), and similar oil yields were observed for the same grape seed samples under industrial conditions.

3.2 Fatty acid composition of grape seed oils

Grape seed oil is rich in polyunsaturated fatty acids. The fatty acid composition of the 8 types of grape seed oil tested is

Table 1. Proportional fatty acid composition of grape seed oils of different cultivars (% mean \pm SD).

Fatty acid	Italian Riesling	Cabernet Franc	Pinot Noir	Sauvignon Blanc	Királyleányka	Rhine Riesling	Merlot	Lemberger
Caprylic acid (C8:0)	0.01 \pm 0.002	0.03 \pm 0.002	0.02 \pm 0.003	0.01 \pm 0.002	0.01 \pm 0.003	n.d.	0.03 \pm 0.005	n.d.
Capric acid (C10:0)	n.d.	0.03 \pm 0.003	0.02 \pm 0.004	n.d.	n.d.	n.d.	0.04 \pm 0.004	n.d.
Lauric acid (C12:0)	0.01 \pm 0.003	0.02 \pm 0.003	0.03 \pm 0.003	0.01 \pm 0.003	0.01 \pm 0.002	0.01 \pm 0.003	0.04 \pm 0.004	0.01 \pm 0.002
Myristic acid (C14:0)	0.07 \pm 0.003	0.10 \pm 0.002	0.10 \pm 0.009	0.07 \pm 0.009	0.05 \pm 0.004	0.08 \pm 0.005	0.07 \pm 0.007	0.07 \pm 0.005
Pentadecanoic acid (C15:0)	0.02 \pm 0.002	0.01 \pm 0.003	0.02 \pm 0.003	0.01 \pm 0.003	0.01 \pm 0.002	0.01 \pm 0.002	0.02 \pm 0.003	0.01 \pm 0.003
Palmitic acid (C16:0)	7.81 \pm 0.12	10.66 \pm 0.01	9.53 \pm 0.04	7.85 \pm 0.15	8.53 \pm 0.21	9.57 \pm 0.09	8.53 \pm 0.11	9.69 \pm 0.09
Palmitoleic acid (C16:1)	0.22 \pm 0.007	0.11 \pm 0.005	0.20 \pm 0.005	0.19 \pm 0.004	0.18 \pm 0.006	0.13 \pm 0.007	0.1 \pm 0.007	0.14 \pm 0.005
Heptadecanoic acid (C17:0)	0.04 \pm 0.002	0.04 \pm 0.004	0.06 \pm 0.004	0.04 \pm 0.004	0.04 \pm 0.003	0.05 \pm 0.002	0.05 \pm 0.005	0.06 \pm 0.003
Heptadecenoic acid (C17:1)	n.d.	n.d.	n.d.	n.d.	0.03 \pm 0.002	0.01 \pm 0.003	n.d.	0.02 \pm 0.002
Stearic acid (C18:0)	4.44 \pm 0.15	4.68 \pm 0.16	4.61 \pm 0.19	4.29 \pm 0.22	3.53 \pm 0.19	9.93 \pm 0.25	3.42 \pm 0.15	3.99 \pm 0.21
Oleic acid (C18:1)	19.92 \pm 0.29	14.29 \pm 0.08	17.52 \pm 0.25	18.79 \pm 0.18	15.16 \pm 0.11	17.91 \pm 0.36	14.49 \pm 0.15	17.18 \pm 0.37
Linoleic acid (C18:2)	66.85 \pm 0.35	69.35 \pm 0.17	67.27 \pm 0.33	68.12 \pm 0.27	71.57 \pm 0.24	67.67 \pm 0.38	72.47 \pm 0.33	68.19 \pm 0.25
Linolenic acid (C18:3)	0.32 \pm 0.009	0.30 \pm 0.003	0.3 \pm 0.008	0.32 \pm 0.003	0.27 \pm 0.003	0.34 \pm 0.007	0.32 \pm 0.006	0.34 \pm 0.007
Arachidic acid (C20:0)	0.13 \pm 0.003	0.20 \pm 0.003	0.15 \pm 0.003	0.12 \pm 0.005	0.09 \pm 0.002	0.13 \pm 0.004	0.15 \pm 0.004	0.15 \pm 0.002
Eicosenoic acid (C20:1)	0.12 \pm 0.004	0.08 \pm 0.007	0.16 \pm 0.007	0.11 \pm 0.009	0.11 \pm 0.005	0.13 \pm 0.004	0.15 \pm 0.003	0.11 \pm 0.008
Docosanoic acid (C22:0)	0.02 \pm 0.003	0.05 \pm 0.008	0.02 \pm 0.002	0.04 \pm 0.003	0.02 \pm 0.002	0.02 \pm 0.003	0.05 \pm 0.006	0.02 \pm 0.002
Lignoceric acid (C24:0)	0.02 \pm 0.002	0.04 \pm 0.004	0.01 \pm 0.003	0.02 \pm 0.003	n.d.	0.01 \pm 0.003	0.07 \pm 0.002	n.d.

Values are means triplicate determination.

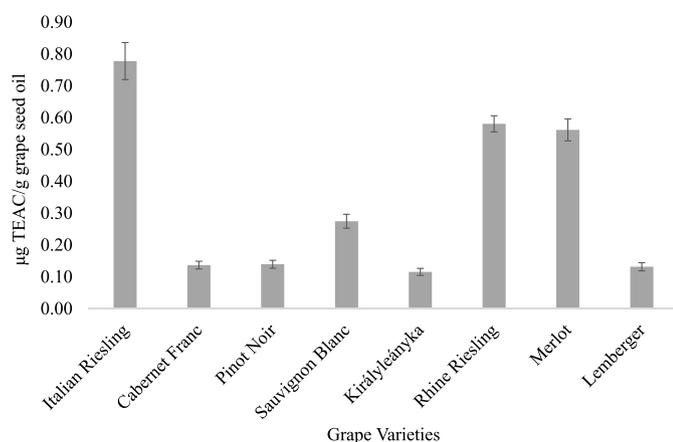


Fig. 2. Antioxidant content of grape seed oils of different cultivars.

shown in Table 1. For all cultivars, the major fatty acids were stearic acid (C18:0), palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C12:2). Linoleic acid was the most abundant fatty acid in all samples, contributing between 66.8% and 72.47% of total fatty acids. The Italian Riesling seeds had the lowest content of linoleic acid (66.85%), with “Merlot” containing the highest amount of 72.47%. The next fatty acid in the row is oleic acid, ranging from 14.29% (Cabernet Franc) to 19.92% (Italian Riesling). The seeds also contained significant palmitic acid. The smallest amount was detected in “Italian Riesling” (7.81%) and the highest amount in “Cabernet Franc” (10.66%). Grape seed oil samples still contained small but significant amounts of stearic acid, the smallest amount of “Merlot” (3.42%), and the largest amount of “Rhine Riesling” (9.93%) contained among the examined grape varieties. The minor fatty acids included caprylic acid, capric acid, lauric acid, myristic acid, pentadecanoic acid, palmitoleic acid, heptadecanoic acid, heptadecenoic acid, linolenic acid, arachidic acid, eicosenoic acid, docosanoic acid, and ignoceric acid (all at < 0.3%). We found significant differences in the fatty acid composition of the oils of each grape variety, a finding supported by several studies (Beveridge *et al.*, 2005; Crews *et al.*, 2006; Pardo *et al.*, 2009; Sabir *et al.*, 2012; Konuskan *et al.*, 2019).

3.3 Antioxidant content of grape seed oils

The antioxidant content of grape seed oils was evaluated using FRAP assay (Fig. 2). Based on the results, it can be stated that the examined grape seed oils showed significant differences. “Italian Riesling” (0.78 µg TEAC/g) had the highest amount of antioxidants, followed by “Rhine Riesling” (0.58 µg TEAC/g), “Merlot” (0.56 µg TEAC/g), and “Sauvignon Blanc” (0.27 µg TEAC/g). As shown in Figure 2, we did not find significant difference between “Rhine Riesling” and “Merlot”. Similar tendency was observed for “Cabernet Franc” (0.14 µg TEAC/g), “Pinot Noir” (0.14 µg TEAC/g), “Királyleányka” (0.12 µg TEAC/g), and “Lemberger” (0.13 µg TEAC/g) (Fig. 2).

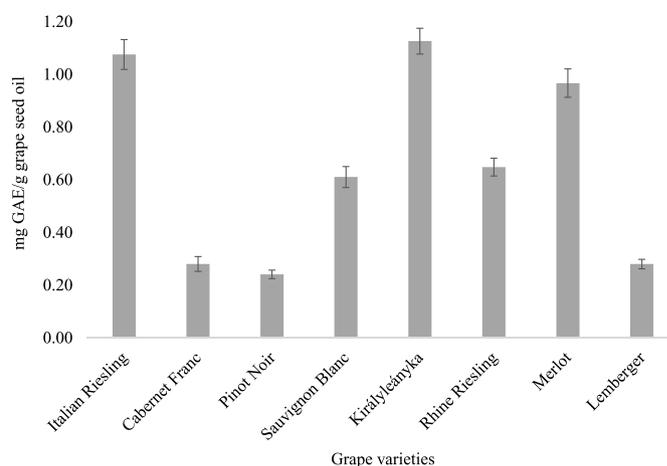


Fig. 3. Phenolic content of grape seed oils of different cultivars.

3.4 Polyphenol content of grape seed oils

Phenolic compounds are poorly soluble in the oily phases, but small amounts are transferred to the oil from the solid matrix during extraction. The method used was able to detect polyphenols from all types of grape seed oil.

The total polyphenol contents of the grape seed oils are shown in Figure 3. Large differences in the total polyphenol content of each grape seed oil variety were observed. The total polyphenol values of the grape seed oils ranged from 0.24 to 1.13 mg GAE/g. The highest polyphenol content was measured for the “Királyleányka” (1.13 mg GAE/g), followed by “Italian Riesling” (1.08 mg GAE/g), “Merlot” (0.97 mg GAE/g), “Rhine Riesling” (0.65 mg GAE/g), “Sauvignon Blanc” (0.61 mg GAE/g), “Cabernet Franc” (0.28 mg GAE/g), “Lemberger” (0.28 mg GAE/g), and “Pinot Noir” (0.24 mg GAE/g).

4 Conclusion

The oil yields of the studied grape varieties showed significant differences and ranged from 9 to 13%. These values fit into the 8–20% oil yields reported in the literature. The fatty acid composition of eight different grape seed oils was determined by GC-MS. As shown by the results obtained, the fatty acid profile of oils was similar. In spite of this, sometimes, we found a significant difference between the fatty acid content of different grape varieties. Our study also revealed that the total polyphenol and antioxidant content of grape seed oil is much lower than that of grape seed, but its consumption can still have a beneficial effect on human health. Our result supported that the analyzed grape varieties are suitable for the production of edible grape seed oil. It is important to note that if we want to press oil under industrial conditions from grape seeds for consumption, we need a large amount of grape seed. In addition to the 8–20% oil yield mentioned in the literature, that means 5–13 kg of dried grape seeds are required to produce 1 liter of grape seed oil.

Conflict of interest

The authors have declared no conflict of interest.

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