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Quality Evaluation of Grape Seed Oils of the Ionian Islands Based on GC-MS and Other Spectroscopic Techniques

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***Abstract*—**Grape seeds are waste products of wineries and often referred to as an important agricultural and industrial waste product with the potential to be used in pharmaceutical, food, and cosmetic applications. In this study, grape seed oil from traditional Ionian varieties was examined for the determination of the quality and the characteristics of each variety. Initially, the fatty acid methyl ester (FAME) profiles were analyzed using Gas Chromatography-Mass Spectrometry, after transesterification. Furthermore, other quality parameters of the grape seed oils were determined by Spectroscopy techniques, UV-Vis and Raman included. Moreover, the antioxidant capacity of the oil was measured by 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assays and their antioxidant capacity expressed in Trolox equivalents. K and ΔK indices were measured in 232, 268, 270 nm, as an oil quality index. The results indicate that the air-dried grape seed total oil content ranged from 5.26 to 8.77% w/w, which is in accordance with the other grape seed varieties tested in similar studies. The composition of grape seed oil is predominated with linoleic and oleic fatty acids, with the linoleic fatty acid ranging from 53.68 to 69.95% and both the linoleic and oleic fatty acids totaling 78-82% of FAMEs, which is analogous to the fatty acid composition of safflower oil. The antioxidant assays ABTS and DPPH scored high, exhibiting that the oils have potential in the cosmetic and culinary businesses. Above that, our results demonstrate that Ionian grape seed oils have prospects that can go further than cosmetic or culinary use, into the pharmaceuticals industry. Finally, the reclamation of grape seeds from wineries waste stream is in accordance with the bio-economy strategic framework and contributes to environmental protection.

***Keywords*—**Antioxidant capacity, fatty acid methyl esters, grape seed oil, GC-MS.

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I. Introduction

GRAPE is the largest fruit crop worldwide, and grape pomace is an important solid waste in wine industry. Wine production is of great importance for the Greek economy, ranking the second most profitable industry of Greece after olives for the years 2015 and 2016 according to FAO statistics [1]. Wine industry is producing respectively large volume solid wastes, which mainly disposed in open areas causing economic and environmental problems although studies are indicating that wineries' wastes can emerge by-products of added value [2], [3].

The wastes from wine making process mainly consist of grape pomace clarification sediment such as lees, and yeast sediment. The grape pomace is the solid material that remains after the pressing, and the fermentation processes consist of pulp, grape seeds and the skins. Grape pomace is of high organic matter, and its environmental release could cause pollution to the adjacent soils and water, unless it is beforehand properly processed. Grape pomace exploitation has been studied for its antimicrobial action, mainly as a potential food preservative [4], [5]. In particular, some phenolic compounds are considered to be the main antimicrobial and antioxidants agents, which are in abundance in the grape skins [6].

A valuable source of phenolic compounds is also found in grape seeds, adding them antioxidant properties as well [7]. Another characteristic of the grape seed constituents is the oily constituents, which are the energy deposits of the seed. Phytosterols, triglycerides and fatty acids are some of them and can be extracted from the seeds by mechanical or solvent means. The extracted oil is rich in poly- and monounsaturated fatty acids (PUFAs and MUFAs), with PUFAs to comprise the majority of the grape seed oil's fatty acids. The content of grape seeds in oil can yield from 5.85 to 22.4 % w/w, and this range is close related to the cultivar or the variety tested, the extraction method used and can also differ from year to year [7]-[9]. It is also known that grape seed oils score high in antioxidant tests. This is of great importance in the food and cosmetic industry and advocates to the emergence of an added value product out of a byproduct [10]-[12]. Agroindustrial waste can offer important nutrient recovery, which are useful as food supplements, cosmetic ingredients or even carriers for other bioactive compounds [12]. At the same time, the wineries' byproducts use is providing an efficient solution to

the management and the reduction of the industrial waste [3].

The goal of this study was the quality evaluation of grape seed oil from some traditional Ionian Islands' grape varieties using different quality parameters in an attempt to determine their potential exploitation as high added value by-products.

II. Materials and Methods

A. Materials

1. Chemicals

All the solvents used were of extra purity (≥99.5%), including, n-hexane, cyclohexane, methanol and ethyl acetate. DPPH and ABTS used for the free radicals' preparation tests, as well as potassium persulphate and Trolox.

2. Plant Material

Grape seeds were provided by Gentillini Wineries and Vineyards, the Robola Cooperative of Cephalonia, Ktima Grampsa and Ktima Theotoky Wineries. Seven of the grape seeds samples were of the variety Robola, six of them came from the protected designation of origin (PDO) of Robola in Cephalonia and the last cultivar came from Zakynthos. The rest of them were of the varieties Tsaousi from Cephalonia, Goustolidi from Zakynthos, Sauvignon Blanc from Cephalonia and Cabernet Sauvignon from Corfu.

B. Methods

1. Oil Extraction from Grape Seeds

a. Moisture Removal

The grape pomace was dried with an air flow ventilation system just after pressing. The moisture content of the pomace was determined, after a constant weight achievement, and grape seeds were separated from the skins with specific diameter sieves, depended on the grape variety. At the end of the drying process, the ratio skins to seeds was specified. Seeds were sealed in polypropylene bags and stored at -20 ^{o}C until their usage.

b. Soxhlet Extraction

After refrigerating, the seeds were left in room temperature for two days. Then, they were pulverized with Philips HR 2074 mixer for 20 seconds in scale two. The crushed grape seed powder was continuously extracted with n-hexane in a Soxhlet apparatus for six hours at a maximum temperature of 70 ^{o}C. The analogy of crushed seeds to n-hexane followed was 1 to 10.

For solvent (n-hexane) removal, a rotary evaporator of reduced pressure was used (Heidolph Laborata 4000), at 40 ^{o} C of bath temperature. The final solvent removal accomplished with nitrogen flow.

2. Determination of FAMEs by GC-MS

The FAMEs were prepared by trans-esterification of oil with 2 N KOH in methanol and n-hexane. Gas chromatographic analysis of FAME performed in a Trace Ultra (Thermo Scientific) gas chromatograph equipped with a TR-5MS column (30 m*0.25 mm I.D., film thickness 0.25 μm), a split injector at 220 °C; detector at 260 °C. Helium was used as carrier gas, with flow ratio 1 mL/min. The program's temperature was: increasing from 110–205 °C at 4 °C min^{-1}, 205-215 °C at 1 °C min^{-1} and final 215-250 °C at 4 °C min^{-1}, where the temperature remained constant for 15 min. The injection volume was 1 μL, in a split-less mode. The identification of FAME was based on the retention times and the mass spectra acquired from DSQII mass spectrometer (Thermo Scientific) and the Adams 0.7 HP, Nist and Xcalibur databases.

3. Antioxidant Activity of Grape Seed Oil

a. DPPH Assay

DPPH assay was used for the radical scavenging activity determination of the grape seed oils. The oil was diluted in ethyl acetate (1:10) and 1 mL of the solution was added to 4 mL of DPPH solution (0.08 mM). Instead of oil, the control was made with ethyl acetate. After 30 minutes in the dark, the absorbance was measured in 515 nm. The ability of the oil to scavenge the DPPH radicals was determined by (1):

$$DPPH scavenging effect\,(\%) = \frac{A_{DPPH} - A_{SAMPLE}}{A_{DPPH}} \times 100 \quad (1)$$

b. ABTS Assay

ABTS assay radical scavenging capacities of the oils are determined based on the capacities of the oils to inhibit ABTS•+ radical. The ABTS•+ was prepared by the reaction of 25 mL of ABTS solution (7 mM) with 440 μl of potassium persulphate (140 mM). The solution left in room temperature for 16-18 hours. The solution was then diluted with ethanol to obtain an absorbance of 0.7± 0.2 at 734 nm. 100 μl of each grape seed oil was mixed with 2 mL of ABTS, and the absorbance was measured after 6 minutes at 734 nm. The antioxidant capacities were obtained by a standard curve of Trolox solutions (0.05-1 mM). The results are expressed in μmol of Trolox equivalents per mL of oil.

4. Spectroscopic Indices (K_{232}, K_{268}, K_{270}, ΔK)

K_{232}, K_{268}, and K_{270} extinction coefficients were measured from the absorption of the samples in the UV region at 232, 268 and 270 nm respectively, with a Cary 60 UV-Vis Agilent spectrophotometer. The samples were prepared by using 1 g of oil in 100 mL cyclohexane and a path length of 10 mm, according to the ISO 3656:2011. ΔK (Delta-K) was calculated by (2):

$$\Delta K = K_{270} - \frac{K_{266} + K_{274}}{2} \quad (2)$$

5. Raman Spectroscopy

Small amount of grape seed oil was injected in glassier capillary tubes until it exceeds the radiation point. Three spectra were acquired using a DeltaNu Raman spectrometer, in 768 nm, for every sample. Integration time was set up for 10 s, and the number of spectra taken each time was settled at 10 captures, with baseline at 160. NuSpec software was used

for visualizing and processing the spectroscopic data.

C. Statistical Analysis

All the assays were performed in triplicate. Final values were calculated as an average of the values from triplicate assay. Data were expressed as means ± S.D.

III. Results and Discussion

The grape seed oil yields' differ for each grape variety tested. Higher yields were assigned to Robola from Zakynthos, yielding 8.77±0.18 w/w, followed by Cabernet Sauvignon from Corfu, 8.11±0.18 w/w. Lower yields were found in cultivars of Robola coming from the PDO of Robola in Cephalonia, ranging from 5.26±0.33 to 7.01±0.85 w/w. Other authors have pointed out that yields are close related to two factors, including the variety tested [12] and the extraction method followed [8].

Grape seed oils' fatty acid composition determined by GC-MS resulted in a rich unsaturated fatty acid profile. Particularly, the FAME composition of the grape seed oils analyzed varied between the different varieties and cultivars. Nevertheless, the abundance of the major (≥0.1%) compounds was in all the samples formed by the same four constituents. Linoleic fatty acid (C18:2) was the most abundant, followed by oleic fatty acid (C18:1), palmitic fatty acid (C16:0) and last, stearic fatty acid (C18:0). Other fatty acids found in the samples in smaller amounts were myristic (C14:0) and palmitoleic (C16:1).

Linoleic fatty acid ranged from 53.28±1.24 to 57.05±1.40% in Robola cultivars coming from the PDO of Robola in Cephalonia. Other varieties appeared to be higher in abundance of linoleic fatty acid. For example, in Tsaousi and Sauvignon blanc from Cephalonia, Cabernet sauvignon from Corfu and Robola from Zakynthos the rates of linoleic fatty acid were 60.95±1.65, 60.82±2.45, 59.66±0.84, and 59.26±0.91, respectively. On the other hand, oleic fatty acid, the second higher in abundance constituent, appeared to be higher in cultivars of Robola from Cephalonia's PDO, with rates starting from 22.41±0.76, and approaching to 25.44±1.36%. High oleic fatty acid content combined with lower linoleic fatty acid rates appears to be a feature common for grape seed oils, as other studies have mentioned [13].

For the rest of the varieties tested, oleic fatty acid was ranged from 17.55±2.37 in Sauvignon blanc from Cephalonia to 21.74±0.28 in Goustolidi from Zakynthos.

The FAMEs dominating the under studied grape seed oils are linoleic and oleic fatty acids. The beforementioned composition has also been reported for well-known industrial oils like safflower oil (originated from normal genotypes) [14] and/or pumpkin oil [15]. As far as other varieties of grape seed oils tested in similar studies, the FAMEs' composition has found to comprise by the same four major fatty acids (linoleic, followed by oleic, palmitic and last in abundance stearic fatty acids) [6]. Pardo et al., in 2009, used the ratio of linoleic and oleic fatty acids as a discriminating parameter between four Spanish grape seed oils [16].

Antioxidant capacity was estimated by ABTS and DPPH assays. Those assays revealed high antioxidant capacities for all sample tested. Those were expressed as Trolox equivalents, by preparing standard curves of 0.1-1 μg/mL of Trolox for DPPH assay (R2=0.99) and 0.05-1.2 μg/mL of Trolox for ABTS assay (R2=0.98). The varieties of Tsaousi from Cephalonia, Goustolidi from Zakynthos and four of the cultivars of the PDO of Robola in Cephalonia exhibited more than 60% scavenging effect of the DPPH radicals, while the beforementioned samples inhibited more than 90% of the effect of the ABTS•+ radical, except in the case of Tsaousi from Cephalonia, where the scavenging effect of the ABTS•+ radical was 64.8 %. The rest of the grape seed oils tested for their antiradical activity showed scavenging effect of the DPPH radicals ranging from 42.95 to 56.25%, while they succeed better in eradicating the ABTS•+, with an inhibition rate from 88.16 to 94.20%. The above results are confirming that antiradical tests are an estimation of the antioxidant capacity of the mixtures given and more than two assays are necessary to evaluate this capacity each time [17]. Furthermore, spectroscopic indices K_{232}, K_{268}, K_{270} and ΔK were measured in the UV region according to the ISO 3656:2011, which is mainly used as a quick test to discriminate virgin olive oils from mixtures with pomace oils, combined to other methods as well, according to EU Regulations for olive oil. In this study, K_{232} was more than 2.50 for three samples, including two of the Robola cultivars of the PDO of Robola in Cephalonia and Tsaousi from Cephalonia. All the other samples' K_{232} were less or equal to 2.50. Moreover, six of the grape seed oils' ΔK were equal to 0.1 and for the other six more than 0.2. According to the EU regulation [18] oils with ΔK above 0.1 are not subjected in the category "extra virgin/ virgin olive oil". K indices can be misleading if used as the only criterion of the oil quality other than olive oil and therefore must be combined with the other quality parameters as well.

Raman spectroscopy was also employed, since it is applicable to every kind of oily samples. It is a technique widely spread for mixtures rich in lipids and is applicable also in a non-destructive way for the samples. The spectra area which has been captured in this study between was 200-2000 cm^{-1}, and another simple evaluation of the oils' unsaturation degree was conducted. The grape seed oils analyzed gave two strong bands, one in 1655 cm^{-1} and the other in 1444 cm^{-1}. Bands in 1655 cm^{-1} are implying a mixture rich in unsaturated cis double bonds, while the band in 1444 cm^{-1} is indicating - CH_2 scissor and twist vibrations, that tend to be stronger as the chain length of the fatty acids increases [19]. There were also smaller bands between 1400 and 800 cm^{-1} that are mainly connected to aliphatic stretches [20].

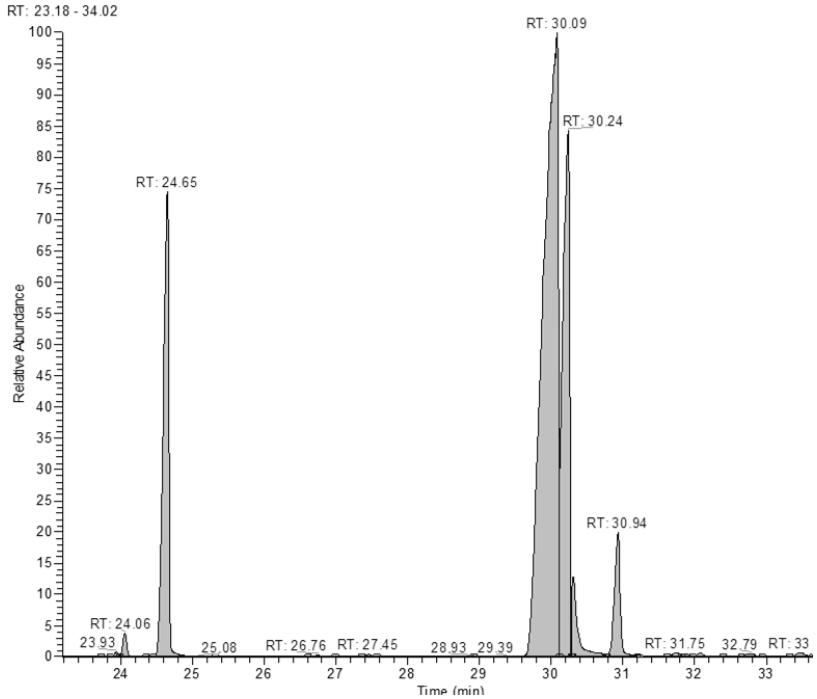

Fig. 1 GC-Chromatograph of Robola grape seed oil. Linoleic fatty acid (C18:2) is the major peak at RT 30.09, followed by oleic fatty acid (C18:1) at RT 30.24, palmitic fatty acid (C16:0) at RT 24.65 and stearic fatty acid (C18:0) at RT 30.94

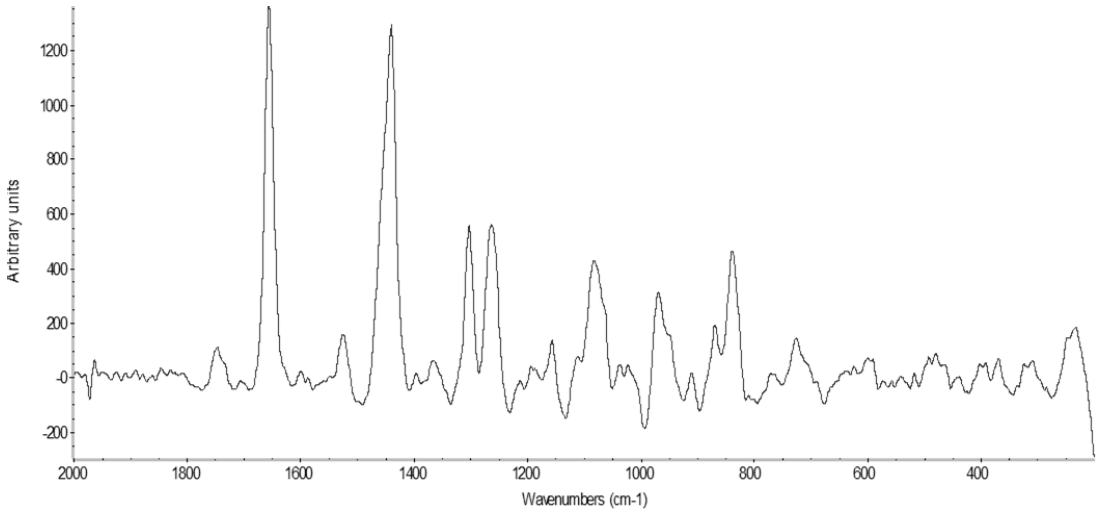

Fig. 2 Raman Spectra of Robola grape seed oil from 200-2000 cm^{-1}. The major peaks in 1655 and 1444 cm^{-1} are of cis double bonds and -CH_2 respectively

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REFERENCES

[1] Food and Agricultural Organization (29 July 2018), FAOSTAT, Retrieved from URL: http://www.fao.org/faostat/en/.

[2] Teixeira, A., Baenas, N., Dominguez-Perles, R., Barros, A., Rosa, E., Moreno, D. A., & Garcia-Viguera, C. (2014). Natural bioactive compounds from winery by-products as health promoters: a review. International journal of molecular sciences, 15(9), 15638-15678.

[3] Da Silva, A. C., & Jorge, N. (2017). Bioactive compounds of oils extracted from fruits seeds obtained from agroindustrial waste. European Journal of Lipid Science and Technology, 119(4), 1600024.

[4] Serra, A. T., Matias, A. A., Nunes, A. V., Leitão, M. C., Brito, D., Bronze, R., Duarte, C. M. (2008). In vitro evaluation of olive-and grape-based natural extracts as potential preservatives for food. Innovative food science & emerging technologies, 9(3), 311-319.

[5] Oliveira, D. A., Salvador, A. A., Smânia Jr, A., Smânia, E. F., Maraschin, M., & Ferreira, S. R. (2013). Antimicrobial activity and composition profile of grape (Vitis vinifera) pomace extracts obtained by supercritical fluids. Journal of Biotechnology, 164(3), 423-432.

[6] Lutterodt, H., Slavin, M., Whent, M., Turner, E., & Yu, L. L. (2011).

Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. Food Chemistry, 128(2), 391-399.

[7] Maier, T., Schieber, A., Kammerer, D. R., & Carle, R. (2009). Residues of grape (Vitis vinifera L.) seed oil production as a valuable source of phenolic antioxidants. Food Chemistry, 112(3), 551-559.

[8] Beveridge, T. H., Girard, B., Kopp, T., & Drover, J. C. (2005). Yield and composition of grape seed oils extracted by supercritical carbon dioxide and petroleum ether: varietal effects. Journal of Agricultural and Food Chemistry, 53(5), 1799-1804.

[9] Sabir, A., Unver, A., & Kara, Z. (2012). The fatty acid and tocopherol constituents of the seed oil extracted from 21 grape varieties (Vitis spp.). Journal of the Science of Food and Agriculture, 92(9), 1982-1987.

[10] Fernandes, L., Casal, S., Cruz, R., Pereira, J. A., & Ramalhosa, E. (2013). Seed oils of ten traditional Portuguese grape varieties with interesting chemical and antioxidant properties. Food Research International, 50(1), 161-166.

[11] Zhao, L., Yagiz, Y., Xu, C., Lu, J., Chung, S., & Marshall, M. R. (2015). Muscadine grape seed oil as a novel source of tocotrienols to reduce adipogenesis and adipocyte inflammation. Food & function, 6(7), 2293-2302.

[12] Rombaut, N., Savoire, R., Thomasset, B., Castello, J., Van Hecke, E., & Lanoisellé, J. L. (2015). Optimization of oil yield and oil total phenolic content during grape seed cold screw pressing. Industrial Crops and Products, 63, 26-33.

[13] Davidov-Pardo, G., & McClements, D. J. (2015). Nutraceutical delivery systems: resveratrol encapsulation in grape seed oil nanoemulsions formed by spontaneous emulsification. Food chemistry, 167, 205-212.

[14] Fernandez-Martinez, J., Del Rio, M., & De Haro, A. (1993). Survey of safflower (Carthamus tinctorius L.) germplasm for variants in fatty acid composition and other seed characters. Euphytica, 69(1-2), 115-122.

[15] Yang, R., Zhang, L., Li, P., Yu, L., Mao, J., Wang, X., & Zhang, Q. (2018). A review of chemical composition and nutritional properties of minor vegetable oils in China. Trends in Food Science & Technology.

[16] Pardo, J. E., Fernández, E., Rubio, M., Alvarruiz, A., & Alonso, G. L. (2009). Characterization of grape seed oil from different grape varieties (Vitis vinifera). European journal of lipid science and technology, 111(2), 188-193.

[17] Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. Analyst, 127(1), 183-198.

[18] European Union Commission: Characteristics of olive and olive pomace oils and their analytical methods. Regulation EC/1989/2003. *Off J Eur Comm*. 2003, L295, 57–66.

[19] Czamara, K., Majzner, K., Pacia, M. Z., Kochan, K., Kaczor, A., & Baranska, M. (2015). Raman spectroscopy of lipids: a review. Journal of Raman Spectroscopy, 46(1), 4-20.

[20] Beattie, J. R., Bell, S. E., Borgaard, C., Fearon, A., & Moss, B. W. (2006). Prediction of adipose tissue composition using Raman spectroscopy: average properties and individual fatty acids. Lipids, 41(3), 287-294.