sustainability

MDPI

Article

Organically Cultivated Vine Varieties—Distinctive Qualities of the Oils Obtained from Grape Seeds

Manuel Alexandru Gitea [1,†], Daniela Gitea [2,*], Delia Mirela Tit [2,3,*], Simona Gabriela Bungau [2,3,†], Mihaela Alexandra Bogdan [2,†], Andrei-Flavius Radu [3], Francisc Vasile Dulf [4] and Manuela Bianca Pasca [2]

[1] Department of Agriculture and Horticulture, Faculty of Environmental Protection, University of Oradea, 410048 Oradea, Romania; giteamanuel@yahoo.com
[2] Department of Pharmacy, Faculty of Medicine and Pharmacy, University of Oradea, 410028 Oradea, Romania; sbungau@uoradea.ro (S.G.B.); mihaela.alexandra.bogdan@gmail.com (M.A.B.); biancapasca28@yahoo.com (M.B.P.)
[3] Doctoral School of Biomedical Sciences, University of Oradea, 410087 Oradea, Romania; andreiflavius.radu@uoradea.ro
[4] Faculty of Agriculture, Department of Environmental and Plant Protection, University of Agricultural Sciences and Veterinary Medicine, 400372 Cluj-Napoca, Romania; francisc.dulf@usamvcluj.ro
* Correspondence: gitea_daniela@yahoo.co.uk (D.G.); dtit@uoradea.ro (D.M.T.)
† Equal contribution to the first author.

Abstract: Grape seeds, which have an increased concentration of high-quality compounds in their oil, are the byproduct of the grape processing industry. The purpose of this study is to evaluate the physico-chemical and bioactive profile of grape seed oil (GSO) obtained by extraction with n-hexane, using three different techniques and coming from two varieties of grapes. DPPH and ABTS radical scavenging ability assessments, and CUPRAC and FRAP assays, were used to determine the oil's antioxidant properties, whereas the total phenolic content (TPC) was determined by applying an adapted version of the Folin–Ciocalteu technique. Utilizing a coupling method of gas chromatography and mass spectrometry, 14 fatty acids have been identified by analyzing their methylated intermediates. GSOs were characterized by a high content of polyunsaturated acids (PUFAs) (69.25–80.32%), of which linoleic acid stands out (66.97 and 79.88%), followed by monounsaturated acids (MUFAs) (16.64–19.59%), with the representative being oleic acid (15.20–17.86%) and then saturated acids (SFAs) (9.26–15.53%), through the palmitic acid (6.29–9.82%). GSO from Merlot samples recovered by MW had the greatest ratio of fatty acids with hypo-/hypercholesterolemia (H/H) values (14.09). The atherogenicity index and thrombogenicity index ranges for red GSO were 0.278–0.393 and 0.242–0.268, respectively, and for white GSO, 0.401–0.440 and 0.256–0.268, respectively. The oil from the red grape variety has the highest quantity of total polyphenols regardless of the extraction method (1.263–2.035 mg GAE/g vs. 0.918–1.013 mg GAE/g). Through the DPPH and FRAP methods, the results were similar (8.443–14.035 µmol TE/g oil and 6.981–13.387 µmol TE/g oil, respectively). The best results were obtained by the CUPRAC method (8.125–19.799 µmol TE/g oil). The assessment of the grape varieties revealed that they are appropriate for making edible GSO, which was endorsed by our results.

Keywords: grape seed oil; ecological culture; bioactive profile; Fetească Regală; Merlot; CUPRAC method; Folin–Ciocalteu method

Citation: Gitea, M.A.; Gitea, D.; Mirela Tit, D.; Bungau, S.G.; Bogdan, M.A.; Radu, A.-F.; Dulf, F.V.; Pasca, M.B. Organically Cultivated Vine Varieties—Distinctive Qualities of the Oils Obtained from Grape Seeds. *Sustainability* **2023**, *15*, 11037. https://doi.org/10.3390/su151411037

Academic Editors: Marc A. Rosen and Vita Di Stefano

Received: 22 May 2023
Revised: 29 June 2023
Accepted: 12 July 2023
Published: 14 July 2023

1. Introduction

One of the oldest and most cultivated plant species is the grapevine (*Vitis vinifera* L.), which is extremely valuable both for its fruits and for the wines that are produced. Creating wine means using about a quarter of the entire world's grape harvest. In the case of Europe, viticulture plays a significant role in the economy of several countries, with France, Italy, and Spain leading the market in the field [1]. Currently, the viticulture field is in continuous

development and modernization, and wine production is one of the most developed industries based on agriculture, experiencing a rapid evolution, especially after the country's accession to the European Union in 2007 [2]. The result was that Romania ranks among the top 15 wine-producing countries in the world [3].

The production of large amounts of byproducts like grape marc, grape seed, grape skin, grape stem, and grape leaf also occurs during the cultivation and processing of grapes. Of these byproducts, a large amount is rich in phenolic chemicals, which are essential to human physiology and used in the food industry, pharmaceutical industry, cosmetics, etc. [4,5]. A significant quantity (10–12%) of the solid byproducts of grape processing are grape seeds [6], where several beneficial substances can be found. However, for a long time, they were mainly burned and used to feed animals, being considered agricultural waste [7].

Grape seed oil (GSO), which is found in quantities between 7–20% in grape seeds, is frequently used, especially in cosmetic formulas, and is abundant in essential fatty acids. Palmitic, linoleic, oleic, and stearic acids make up much of the fatty acid composition of GSO. Also, GSO contains one of the most abundant naturally occurring forms of tocols, especially tocopherols, which are highly potent oil-soluble antioxidants. Due to its nutritional benefits and favorable sensory qualities, this oil is currently very common for consumption. The high concentration of important fatty acids, natural antioxidants, and phytochemicals in this oil makes it both a helpful dietary supplement and a cosmetic product [8]. Furthermore, antioxidants provide an essential defense mechanism against oxidative damage and play a key role in contributing to the improvement of the management of numerous diseases [9].

Phenols are organic compounds that have antioxidant properties and are found in grapes, particularly in the seeds and extracts of grapes. Anthocyanins, proathocyanidins, flavonols, and flavan-3-ols (that are part of the flavonoid family), as well as stilbenes and phenolic acids (which are not in the flavonoid family), are some of the most significant grape polyphenols. The various families can exist in either conjugated or free forms, and each one is distinct from the others in terms of the degree of hydroxylation, the way the hydroxy groups are substituted (glycosylation, methylation, or acylation), and even creating adducts between them (e.g., condensed tannins; anthocyanins with phenolic acids). These data clarify why grape polyphenols have such a wide chemical variety [10,11]. GSO's water-soluble phenolic content is relatively low; however, using the right oil extraction techniques can raise the phenol level of oils [12]. By using colorimetric techniques and standard curves generated after testing, known quantities of isolated polyphenol molecules, like gallic acid or catechin, and the total amount of polyphenols can be determined [13]. Total seed polyphenols are usually determined by colorimetric methods with Folin–Ciocalteu reagent [14,15]. The ability of an antioxidant to counteract oxidation is determined by the Folin–Ciocalteu reaction, a test that relies on electron transfer [16]. It is frequently used to determine the amount of total phenol/polyphenol contained in foods produced from plants and biological specimens [17].

The methods of extracting GSO that are the most frequently used are pressing and methods involving organic solutions (Bligh and Dyer or Soxhlet). Although solvent extraction produces a better yield, it has the disadvantages of a longer processing time, the presence of potentially harmful residues in the finished product, and reduced nutritional properties of the oil. Cold extraction of oils is frequently linked with decreased final production [18]. Pressurized liquid extraction, microwave (MW)-assisted extraction, ultrasound (US)-assisted extraction, and supercritical fluid extraction (SFE), which employs fluids and CO_2, are additional techniques for extracting oil. US extraction uses negative pressure after US treatment, while MW extraction employs nonionizing electromagnetic waves that are converted to thermal energy. Both methods target cell wall destruction to make extraction easier [19,20].

Through this research, we wanted to highlight and provide the distinctive qualities (physicochemical characteristics, fatty acid content, antioxidant potential, functional value, and total phenolic content) of GSO obtained from two varieties of vines, namely Feteasc̆a

Regală (a Romanian-specific white variety) and Merlot (an international red variety), cultivated in an ecological culture system. GSOs have been extracted with n-hexane using three different techniques (US-assisted extraction, cold extraction under stirring, and MW-assisted extraction). According to our knowledge, there are very few studies [21] that characterize the GSO obtained from Fetească Regală, and our research is unique considering the approach. The results offer new perspectives and research opportunities for the incorporation of these extracts into different functional foods, pharmaceuticals, or cosmetic products.

2. Results

2.1. Macroscopic and Microscopic Analyses

The macroscopic analysis shows that the two varieties of *Vitis vinifera* L. differ in the color of the fruits, size, and number of seeds. In the Fetească Regală variety (Figure 1), the seeds are pyriform in shape and dark brown in color. The surface is smooth with a ridge on the back, the tip is discoidal, the size is 4–8 mm long, and the taste is bitter. In most grapes, we find two seeds in one fruit. In the Merlot variety, the shape of the seeds is still pyriform, the color is dark brown, the surface is smooth, with a ridge on the back surface, the tip is discoidal, the size is 4–6 mm long, and the taste is bitter. Most fruits have three seeds.

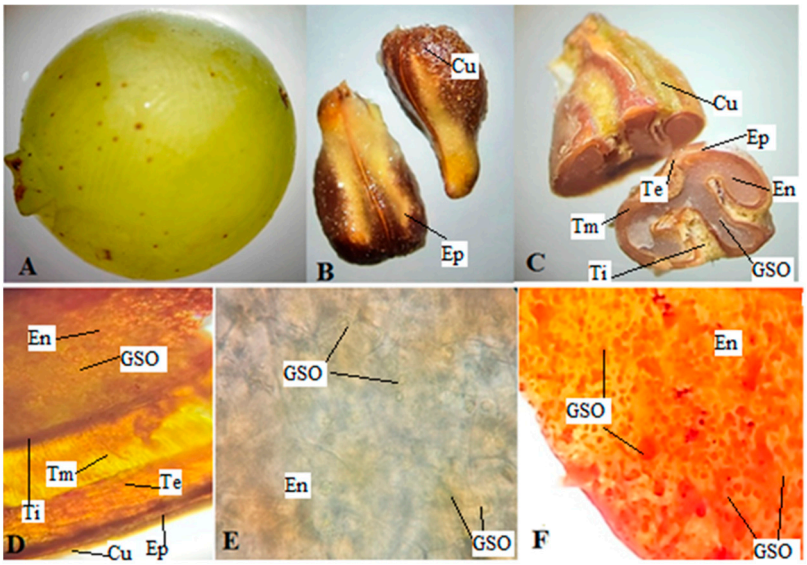

Figure 1. *Vitis vinifera* L.—Fetească Regală (**A**)Fruit; (**B**) Seeds (kernels); (**C**) Transverse section of seeds (ob.4×); (**D**) Transverse section of seed showing regions of cuticle, epidermis, outer integument, mid-integument, and inner integument (ob.10×); (**E**,**F**)Transverse section through the endosperm, highlighting the oil droplets (ob.40×); Cu, cuticle; Ep, epidermis; Te, external integument; Ti, intertegument; Tm, medium integument; En, endosperm; GSO, grape seed oil.

The microscopic analysis was carried out on cross-sections through the seeds of both varieties, highlighting five areas: cuticle and epidermis; outer integument or soft seed coat, composed of parenchyma tissue; middle integument or hard seed coat, composed of two layers of cells; inner skin; endosperm and embryo (Figure 2a–f). A thin cuticle that is not very developed can be observed, followed by the epidermis made up of rectangular cells. The outer skin is made up of parenchymal cells. The epidermis and outer integument form a soft layer of cells that covers the seed. Two layers of rectangular, thin-walled cells follow, which constitute the middle tissue or the middle integument. The inner integument lies between the mid-integument and the endosperm. The center of the seed consists of parenchymatous tissue, surrounded by integuments and containing the embryo sac. The presence of oil droplets in all the parenchymal tissues that make up the endosperm is highlighted by the staining technique [22].

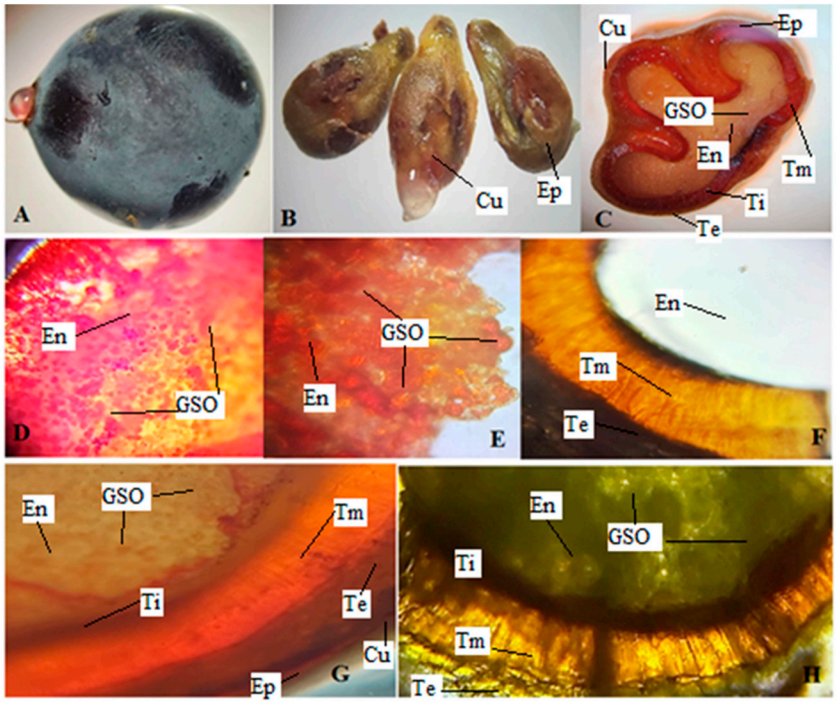

Figure 2. *Vitis vinifera* L.—*Merlot* (**A**) Fruit; (**B**) Seeds (kernels); (**C**) Transverse section of seeds (ob.4×); (**D**) Transverse section through the endosperm, highlighting the oil droplets (ob.40×); (**E**–**H**) Transverse section of seed showing regions of cuticle, epidermis, outer integument, mid-integument, and inner integument (ob.40×). Cu, cuticle; Ep, epidermis; Te, external integument; Ti, inter tegument; Tm, medium integument; En, endosperm; GSO, grape seed oil.

2.2. Extraction Yield and Physical Indices

An extraction yield has been determined for each GSO extraction based on the dried material in grams. The highest yield was obtained through solvent extraction method by stirring at room temperature (11.67–12.52%), followed by MW (10.52–10.79%) and then US extraction (9.02–10.73). The yield was significantly different ($p < 0.05$), depending on the extraction method used, for all samples (Table 1). GSOs have a medium density (0.8732–0.9147 g/mL).

Table 1. Physico-chemical characterization of GSOs and extraction yields.

GSO Sample	Amount of Seed/Solvent	Oil Quantity (g)	Oil Volume (mL)	Density (g/mL)	Yield (% *w*/*w*)
GSO_M_US	30 g/250 mL	2.707 ± 0.01 ^{a}	3 ± 0.10 ^{a}	0.9023 ± 0.02 ^{a}	9.02 ± 0.05 ^{a}
GSO_M_stirring		3.503 ± 0.12 ^{b}	3.7 ± 0.20 ^{b}	0.9467 ± 0.02 ^{a}	11.67 ± 0.38 ^{b}
GSO_M_MW	20 g/400 mL	2.157 ± 0.04 ^{c}	2.3 ± 0.10 ^{b}	0.937 ± 0.03 ^{a}	10.79 ± 0.20 ^{c}
GSO_FR_US	30 g/250 mL	3.220 ± 0.08 ^{a}	3.6 ± 0.10 ^{a}	0.8944 ± 0.04 ^{a}	10.73 ± 0.27 ^{a}
GSO_FR_stirring		3.755 ± 0.16 ^{b}	4.3 ± 0.10 ^{b}	0.8732 ± 0.04 ^{a}	12.52 ± 0.52 ^{b}
GSO_FR_MW	20 g/400 mL	2.104 ± 0.10 ^{c}	2.3 ± 0.10 ^{c}	0.9147 ± 0.03 ^{a}	10.52 ± 0.50 ^{c}

GSO, grape seed oil; M, Merlot; US, ultrasound; MW, microwave; FR, Feteasca Regală. Data are reported as mean ± SD; all determinations were made in triplicate; ^{a,b,c,} significant difference between data for GSO obtained from the same variety by three different methods (US, stirring, MW), for one test, by applying Tukey's test for $p < 0.05$. Results from a wide range of sources show no statistically different means for superscripts with the same letter.

The oil extracted from the grape seeds has a yellowish, brown-yellow, or yellow greenish color (Figure 3).

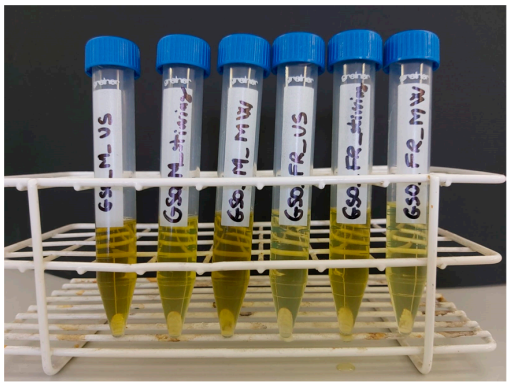

Figure 3. Grape seed oil samples.

2.3. Fatty Acid Composition and Functional Quality

Monounsaturated fatty acids (16.64–19.59%), saturated fatty acids (9.26–15.53%), and polyunsaturated fatty acids (67.37–80.32%) were all present in the samples, as shown by the findings presented in Table 2. The most common saturated fatty acid was palmitic acid (6.29–9.82%), which was followed by stearic acid (2.75–5.32%). The percentage of monounsaturated fatty acids ranged from 15.20% to 17.86% of total fatty acids. In the studied samples, linoleic acid was the most prevalent polyunsaturated fatty acid, making up between 66.97 and 79.88% of the total fatty acid content. No matter the grape variety, or the extraction method, a total of 14 fatty acids were found in all samples. For the oil obtained from the Merlot variety, ΣSFAs were significantly different ($p < 0.05$), depending on the extraction method, with the lowest quantity being obtained by MW (Table 2).

Table 2. Fatty acid concentrations (%) in white and red GSOs, according to various extraction methods, expressed as percentages.

Fatty Acids		Rt	(M+)	GSO_M_US	GSO_M_ Stirring	GSO_M_ MW	GSO_FR_ US	GSO_FR_ Stirring	GSO_FR_ MW
(6:0)	Hexanoic	7.804	130	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.02 ± 0.01	0.07 ± 0.01	0.05 ± 0.00
(14:0)	Myristic	18.308	242	0.03 ± 0.00	0.06 ± 0.01	0.02 ± 0.00	0.07 ± 0.01	0.05 ± 0.00	0.07 ± 0.01
(16:0)	Palmitic	21.224	270	8.26 ± 0.71	9.82 ± 0.10	6.29 ± 0.70	8.78 ± 0.9	8.69 ± 0.90	9.48 ± 0.75
(18:0)	Stearic	24.46	298	4.66 ± 0.38	5.32 ± 0.50	2.75 ± 0.30	4.07 ± 0.40	4.15 ± 0.03	4.04 ± 0.30
(20:0)	Arachidic	28.852	326	0.23 ± 0.01	0.23 ± 0.02	0.13 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.17 ± 0.01
(22:0)	Behenic	35.515	354	0.05 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
	ΣSFAs	-	-	13.30 ± 0.21 [a]	15.53 ± 0.37 [b]	9.26 ± 0.52 [c]	13.15 ± 0.71	13.17 ± 0.45	13.90 ± 1.03
16:1(n-9)	cis-7 hexadecenoic	21.579	268	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.08 ± 0.00	0.05 ± 0.01	0.08 ± 0.01
16:1(n-7)	Palmitoleic	21.674	268	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.20 ± 0.15
18:1(n-9)	Oleic	24.97	296	15.84 ± 1.04	17.86 ± 1.46	15.20 ± 0.90	15.79 ± 1.40	15.58 ± 1.35	17.37 ± 1.50
18:1(n-7)	Vaccenic	25.075	296	1.20 ± 0.95	1.29 ± 1.5	0.88 ± 0.05	0.81 ± 0.07	0.79 ± 0.15	0.89 ± 0.10
20:1(n-9)	11-eicosenoic	25.075		0.25 ± 0.01	0.27 ± 0.02	0.41 ± 0.03	0.24 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
	ΣMUFAs	-	-	17.45 ± 0.32	19.59 ± 0.77	16.64 ± 0.45	17.08 ± 0.41	16.75 ± 0.53	18.73 ± 0.16
18:2(n-6)	Linoleic	26.051	294	68.74 ± 5.30	79.88 ± 8.14	73.72 ± 6.40	69.40 ± 6.01	69.76 ± 5.60	66.97 ± 5.03
20:2(n-6)	Eicosadienoic	31.088	322	0.06 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	0.05 ± 0.00	0.05 ± 0.00	0.07 ± 0.00
18:3(n-3)	α-linolenic	27.446	292	0.45 ± 0.01	0.40 ± 0.01	0.34 ± 0.02	0.33 ± 0.02	0.28 ± 0.01	0.34 ± 0.02
-	ΣPUFAs	-	-	69.25 ± 0.46	80.32 ± 0.51	74.10 ± 0.35	69.77 ± 0.12	70.09 ± 0.42	67.37 ± 0.26
-	n-3	-	-	0.45 ± 0.01	0.40 ± 0.01	0.34 ± 0.02	0.33 ± 0.02	0.28 ± 0.01	0.34 ± 0.02
-	n-6	-	-	68.80 ± 0.57	79.93 ± 0.30	73.75 ± 0.18	69.45 ± 0.32	69.81 ± 0.22	67.03 ± 0.30
-	n-3/n-6	-	-	0.0065	0.005	0.0046	0.0047	0.004	0.005
-	PUFAs/SFAs	-	-	5.21	5.17	8.00	5.31	5.32	4.85

GSO, grape seed oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Rt, retention time of the methyl esters of the fatty acids; M+, Molecular ion of the methyl esters of the fatty acids; [a,b,c,] significant difference between data, for one test, by applying Tukey's test for $p < 0.05$. Data are reported as mean ± SD; all determinations were made in triplicate; US, ultrasound; MW, microwave.

Figure 4 presents the GC-MS chromatogram for the fatty acid profile of grape seed oil obtained by stirring.

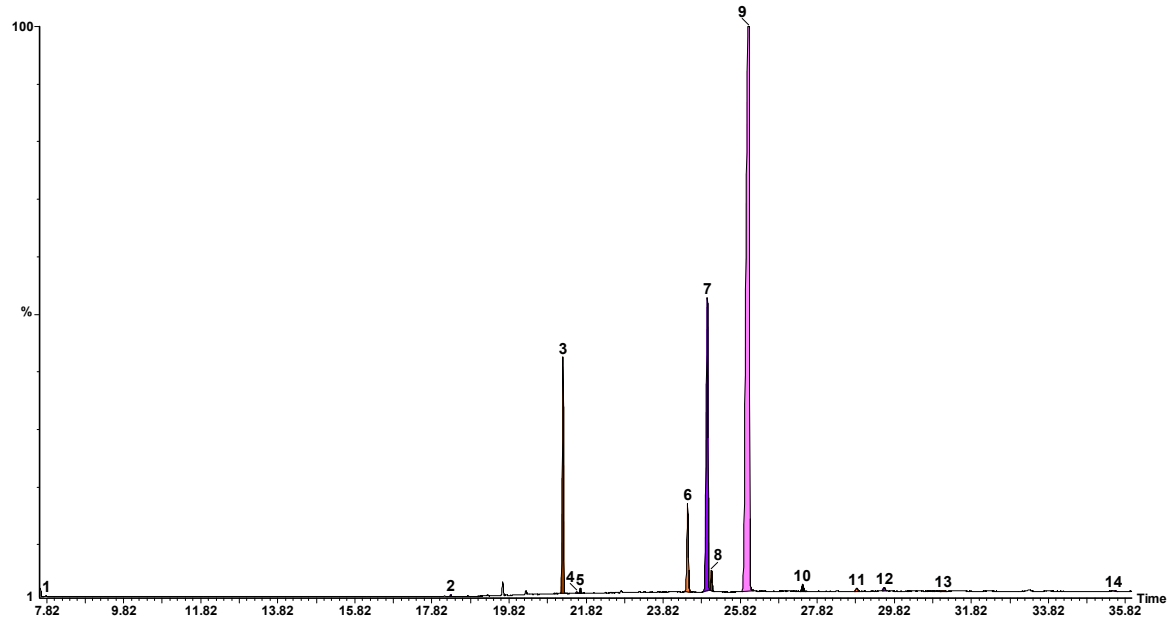

Figure 4. Chromatogram obtained by gas chromatography–mass spectrometry for the methylated derivatives of fatty acids of grape seed oil obtained by stirring from Fetească Regală. 1, (6:0); 2, (14:0); 3, (16:0); 4, 16:1(n-9); 5, 16:1(n-7); 6, (18:0); 7, 18:1(n-9); 8, 18:1(n-7); 9,1 8:2(n-6); 10, 18:3(n-3); 11, (20:0), 12, 20:1(n-9), 13, 20:2(n-6); 14, (22:0).

The analysis of GSO functional characteristics may benefit from taking into account the fatty acid chemical profile. As only three SFAs are hypercholesterolemic, the polyunsaturated/saturated fatty acids ratio (PUFA/SFA) is frequently used to assess indicators for common symptoms of cardiovascular impairments (thrombogenicity and atherogenicity) [23]. Therefore, we determined the atherogenicity index (AI), thrombogenicity index (TI), and hypo- and hypercholesterolemic fatty acid ratios (H/H). Red GSOs' H/H values varied from 9.89 to 14.09, showing significant differences ($p < 0.01$) between the three samples obtained by using the extraction methods. White GSOs' H/H values ranged from 8.83 to 9.76, with significant differences between the oil obtained by MW compared to those obtained by stirring and US. Atherogenicity index AI range for red GSO was 0.278–0.393, and for white GSO, it was 0.401–0.440. Red GSO thrombogenicity index (TI) rates ranged from 0.242–0.268, and white GSO from 0.256–0.268. AI and TI values did not differ significantly, regardless of the extraction method (Table 3).

Table 3. GSO functional quality indicators derived from various extraction methods.

Sample	H/H	AI	TI
GSO_M_US	10.20 ^{a}	0.381 ^{a}	0.291 ^{a}
GSO_M_stirring	9.89 ^{b}	0.393 ^{a}	0.298 ^{a}
GSO_M_MW	14.09 ^{c}	0.278 ^{a}	0.196 ^{a}
GSO_FR_US	9.63 ^{a}	0.404 ^{a}	0.219 ^{a}
GSO_FR_stirring	9.76 ^{a}	0.401 ^{a}	0.292 ^{a}
GSO_FR_MW	8.83 ^{b}	0.440 ^{a}	0.309 ^{a}

AI, atherogenicity index; TI, thrombogenicity index; H/H: ratio between hypocholesterolemic and hypercholesterolemic fatty acids; ^{a,b,c,} significant difference between data for GSO obtained from the same variety by three different methods (US, stirring, MW), for one test, by applying Tukey's test for $p < 0.05$.

2.4. Determination of Total Phenolic Content

The GSOs' overall polyphenol content ranged from 0.918 to 2.035 mg GAE/g, with significant variations depending on the extraction method only in the case of GSOs from Merlot. GSO_M_MW had the highest content of polyphenols, significantly different from GSO_M_stirring and GSO_M-US (2.035 vs. 1.533 mg and 1.263 mg GAE/g GSO_M_US, $p < 0.01$, respectively).

In GSOs from Fetească Regală, the highest content of polyphenols was recorded in the oil obtained by stirring, which was insignificantly higher compared to the other samples (1.013 mg GAE/g vs. 0.938 mg GAE/g GSO_FR_US and 0.918 MW, $p > 0.05$, respectively). The Merlot variety polyphenol content was found to be significantly higher ($p < 0.01$), regardless of the extraction method (Figure 5).

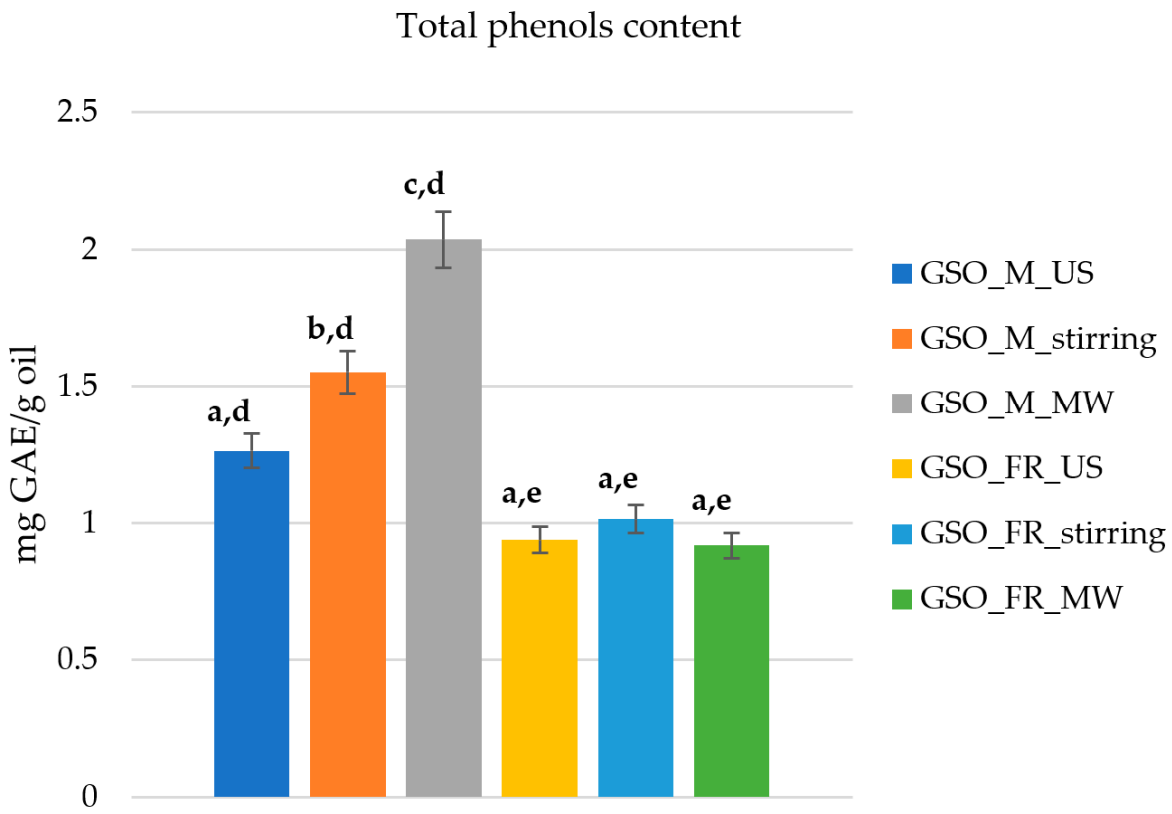

Figure 5. Determination of the total phenolic content (mg GAE/g oil) of grape seed oils obtained by Ultrasound-Assisted Extraction (US), Conventional Extraction (stirring), and Microwave-Assisted Extraction (MW), from the Merlot and Feteasca Regală varieties. a, b, c, significant difference between data for GSO obtained from the same variety by three different methods (US, stirring, MW); d, e, significant difference between data for GSO obtained from each variety by the same methods (US, stirring, MW), for one test, by applying Tukey's test for $p < 0.05$.

2.5. Antioxidant Capacity Determination of Grape Seed Oils

The highest antioxidant capacity was found in GSO from Merlot, obtained by MW extraction. Through the DPPH and FRAP methods, the results were similar. Using DPPH, we obtained values between 8.443–14.035 µmol TE/g oil, and the FRAP method revealed values between 6.129–13.387 µmol TE/g oil. The best results were obtained by the CUPRAC method, with values between 5.993–19.799 µmol TE/g oil, while the worst results were obtained through ABTS (1.124–4.025 µmol TE/g oil) (Table 4).

The ABTS method, commonly used as an electron acceptor in various tests to measure antioxidant activity, can be used in the analysis of GSO but with lower results than other methods.

Table 4. Antioxidant capacity of grape seed oils.

GSO Sample	DPPH (µmol TE/g Oil)	FRAP (µmol TE/g Oil)	CUPRAC (µmol TE/g Oil)	ABTS (µmol TE/g Oil)
GSO_M_US	8.553 ± 0.076 [a]	9.677 ± 0.913 [a]	13.498 ± 2.849 [b]	4.025 ± 0.205 [a]
GSO_M_stirring	14.035 ± 0.554 [b]	6.921 ± 0.193 [b]	9.840 ± 2.799 [b]	1.595 ± 0.079 [b]
GSO_M_MW	10.307 ± 0.526 [c]	13.387 ± 0.374 [c]	19.799 ± 0.733 [c]	1.908 ± 0.134 [c]
GSO_FR_US	7.675 ± 0.080 [a]	1.040 ± 0.270 [a]	5.993 ± 0.441 [a]	2.768 ± 0.107 [a]
GSO_FR_stirring	8.443 ± 0.225 [b]	6.129 ± 0.170 [b]	14.636 ± 0.330 [b]	1.124 ± 0.056 [b]
GSO_FR_MW	7.905 ± 0.021 [a]	6.981 ± 0.120 [c]	8.125 ± 0.022 [c]	1.255 ± 0.025 [b]

GSO, grape seed oil; DPPH, 2,2′-diphenyl-1-picrylhydrazyl radical; FRAP, ferric reducing ability of plasma; CUPRAC, cupric ion reducing antioxidant capacity; ABTS, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid. Data are reported as mean ± SD; all determinations were made in triplicate; [a,b,c,] significant difference between data for GSO obtained from the same variety by three different methods (US, stirring, MW), for one test, by applying Tukey's test for $p < 0.05$. Results from a wide range of sources show no statistically different means for superscripts with the same letter.

The Pearson correlation matrix was used to assess the connection between the antioxidant capability and total phenol content (TP) of GSO samples. TP was positively and significantly correlated with antioxidant capacity measured by FRAP, DPPH, and CUPRAC for most samples. No correlation was observed between TP and antioxidant capacity, regardless of the method, for oil obtained from the Fetească Regală variety by MW extraction. Negative non-significant correlations were obtained between TP and ABTS for oils obtained from the Merlot variety by US extraction and the Fetească Regală variety by US and MW (Table 5).

Table 5. Pearson correlation coefficients of TPC and antioxidant capacity of GSO samples.

Antioxidant Capacity	Pearson Correlation	GSO_M_US	GSO_M_ Stirring	GSO_M_ MW	GSO_FR_ US	GSO_FR_ Stirring	GSO_FR_ MW
FRAP	r	**1.000 ***	**1.000 ***	**1.000 ***	**1.000 ***	**1.000 ***	0.991
	p	**0.002**	**0.008**	**0.003**	**0.019**	**0.010**	0.085
DPPH	r	0.993	**1.000 ***	**1.000 ***	**1.000 ***	**1.000 ***	0.524
	p	0.076	**0.005**	**0.003**	**0.017**	**0.006**	0.649
CUPRAC	r	**1.000 ***	**1.000 ***	**1.000 ***	**1.000 ***	0.871	−0.989
	p	**0.003**	**0.005**	**0.002**	**0.019**	0.327	0.096
ABTS	r	−0.712	0.222	0.453	−0.978	0.196	−0.704
	p	0.495	0.857	0.700	0.134	0.874	0.503

* Correlation is significant at the 0.005 level (2-tailed); bolded values, significant values. TP, total polyphenols; r, Pearson coefficient; US, ultrasound; MW, microwave.

3. Discussion

Since a very large amount of seeds is produced worldwide, considering the beneficial effect of consuming GSO on health and the possible uses in other industries, the extraction of oil from grape seeds (*V. vinifera* L.) is presently the widest use of these seeds [24]. An important role in the safety of the use of products, which contain active principles from plants, is attributed to the source and quality of the plant material. Many factors can affect the quality and, consequently, the value of phyto-complexes, such as light exposure, temperature, water availability, nutrients, collection period and time, collection method, drying, packaging, storage, and transportation of raw materials. For this reason, it is of utmost importance to have raw materials with consistent and reproducible quality standards [25].

In this study, GSOs obtained from two varieties of grapes have been analyzed, a red one (Merlot, an international variety) and a white one (Fetească Regală, Romanian variety), obtained from an ecological vine culture, with all the grapes being harvested at maturity.

The seeds were analyzed from the point of view of their macro-/microscopic characteristics to identify the potential differences between the two varieties. As far as we know, this type of sections analysis was performed for the first time in this study for the varieties we are referring to. The microscopic analysis of the cross-sections through the grape seeds did not reveal any differences between the two varieties. The presence of oil drops in the endosperm was located/highlighted.

According to earlier research, the percentage of oil in grape seeds is between 6% and 20% [26–28]. The chemical composition is primarily influenced by the stage of seed maturation, various environmental cultivation conditions, and to a lesser extent, the seed extraction procedure [6,29].

In our study, the oil was extracted using novel methods like MW, US, and cold extraction, using n-hexane as a solvent. These techniques present the advantage of shorter extraction times and lower temperatures, which slow down the degradation of thermolabile oily components during the procedure [23]. The yield of GSO extraction varied slightly depending on the extraction method and grape varieties. The highest yield was obtained through the solvent extraction method by stirring at room temperature, followed by MW and then US extraction. This order is kept for both grape varieties.

Moreover, it must be mentioned that the yield of GSO extraction can vary depending on several factors, such as the type of grapes, the growing conditions, the extraction method used, and the quality of the grape seeds. However, on average, the yield of GSO extraction can vary greatly, and in some cases, it can be 10–15% or 25–30% [30,31]. The use of an organic solvent, which has been removed by evaporation, ensures a better yield for oil extraction than the use of a press, where the extraction temperature cannot be controlled, being quite high due to the pressing force [30,31]. Although no prior studies to support the MW technique's suitability for isolating oils from grape seeds were found in the literature, Dimić et al. compared three methods of obtaining GSO and found that the MW technique demonstrated excellent extraction yield [23]. Our findings thus support the utilization of MW for this objective.

An essential factor in determining a vegetable oil or fat's nutritional value and potential for industrial use is the quantification of each fatty acid. The varieties and proportions of fatty acids in vegetable oils have a significant impact on their physicochemical and nutritional properties [32]. It is important to observe that cultivation conditions and grape variety may have a significant impact on the fatty acid composition of GSOs [24].

In the present study, the profile of fatty acids is similar to that found in data from the literature [23]. The oil obtained from the two grape varieties, regardless of the extraction technique, is characterized by a high content of polyunsaturated acids (PUFAs) (69.25–80.32%), of which linoleic acid stands out (66.97 and 79.88%), followed by monounsaturated acids (MUFAs) (16.64–19.59%), with the representative being oleic acid (15.20–17.86) and then saturated acids (SFAs) (9.26–15.53%), through the palmitic acid (6.29–9.82%). According to earlier research, PUFAs predominated in the grape seed samples with concentrations of 69.27–74.88%, followed by MUFAs, varying between 13.53–18.62%, and SFAs with concentrations from 11.28 to 12.27%. Considering the fatty acid profiles, stearic acid, which varied from 3.79 to 4.37%, followed palmitic acid, which dominated the category of SFAs with 7.20–7.93%. Oleic acid, which made up 13.39–18.47% of the MUFA contents, was identified as the main acid from the samples [23].

Regardless of the variety or the extraction technique, linoleic acid was discovered to be the most prevalent fatty acid among those discovered, contributing 68.74% to 79.88% in the Merlot variety and 66.97 to 69.76% in the Fetească Regală variety. Viktória Kapcsándi et al. [12], using the Soxhlet extraction method with petroleum ether as a solvent for 3 h, obtained from the Merlot variety an oil with a linoleic acid content of 72.47%, a value close to the linoleic acid content of the oil obtained by us simply by shaking the powder from

grape seeds of the Merlot variety with n-hexane (79.88%). Additionally, Dilsat Bozdogan Konuskan et al. examined total phenolic contents, the fatty acid profiles, and antioxidant activity of the oil obtained from grape seeds of the Merlot variety and other varieties using solvent and cold-pressed acquisition techniques. The GSOs obtained through solvent extraction had the greatest concentrations of linoleic acid, the most prevalent fatty acid, antioxidant activity, and total phenolic content [33]. GSO is an oil with high linoleic acid content. Due to its emollient properties, linoleic acid has benefits in moisturizing and strengthening the skin's protective barrier [24].

The data on functional quality indices showed that GSOs from Merlot samples recovered by MW had the greatest H/H values (14.09). Since this index shows how fatty acids affect cholesterol metabolism, a higher level is preferred in nutrition. For example, beneficial oils like sesame and olive oils have lower values than linseed, which has a similar H/H index to grape oils (13.24) [34]. The AI and TI ranges for red GSO were 0.278–0.393 and 0.242–0.268, respectively, and 0.401–0.440 and 0.256–0.268, respectively, for white GSO. Since they result in oil with an excellent functional and nutritional composition, AI and TI values near zero are preferred. Reduced AI and TI have an impact on preventing coronary diseases [35]. Dimić et al. found that white and red GSOs had an average AI of 0.085, which is less than our results report [23].

Although phenolic compounds are poorly soluble in oily phases, extraction does transfer a tiny quantity of them from the solid matrix to the oil [36]. In our study, we observed that the oil obtained from the red grape variety has the highest number of total polyphenols regardless of the extraction method (1.263–2.035 mg GAE/g vs. 0.918–1.013 mg GAE/g). According to earlier research by Kapcsándi et al., the overall polyphenol content of GSOs ranged from 0.24 to 1.13 mg GAE/g for Merlot being 0.97 mg GAE/g, lower than in this study [12].

DPPH and ABTS radical scavenging ability assessments, CUPRAC and FRAP assays, as well as other tests were used to determine the oil's antioxidant properties. These tests were all carried out three times to ensure their reproducibility and to obtain a more complete picture of the oil's antioxidant activity. DPPH and FRAP are chemical compounds used as standards in antioxidant tests to determine the antioxidant capacity of various substances, including GSO. Through the DPPH and FRAP methods, the results were similar (8.443–14.035 µmol TE/g oil and 6.981–13.387 µmol TE/g oil, respectively). The best results were obtained by the CUPRAC method (8.125–19.799 µmol TE/g oil). The ABTS method, commonly used as an electron acceptor in various tests to measure antioxidant activity, showed the lowest results (1.124–4.025 µmol TE/g oil). Mollica et al. came to a different conclusion, indicating that the oil produced from the Montepulciano variety exhibited minimal action in various antioxidant bioassays and no activity in the DPPH and ABTS assays [37]. But through the method used, we demonstrated the antioxidant action of GSO, with the oil obtained from the Merlot variety having the best antioxidant activity. The results could vary because each grape variety contains a different type of phenolic compound or because these compounds have been associated with other compounds, making them more complex or insoluble, like long chains of cutin and suberin, which prevent a direct reaction with the DPPH radical [38].

Antioxidant capacity and total polyphenol concentration showed a strong and favorable correlation, except for ABTS, for most samples, regardless of the method of extraction and variety.

It is undeniable that oil making constitutes an advantageous and sustainable utilization of grape seeds considering the wine sector's continuous waste growth, as well as all the GSO's benefits for health. According to the findings of the current research, GSO is an important source of constituents with antioxidant activity. Furthermore, this investigation highlights a characteristic of a Romanian indigenous grape variety that has not yet received enough attention. We must emphasize, as a strong point, the novelty of the study carried out on an organic native vine crop (Feteasc̆ă Regală), with the limitation consisting in the lack of detailing of the phenolic profile and the fact that solvent residues were not analyzed.

4. Materials and Methods

4.1. Description of the Plant Material

The vineyard from which the plant material was obtained is part of the vineyard area of the Crişana and Maramureş Wine Regions, Bihor County, Romania, located at 47°16′10.1″ N 22°08′04.2″ E, and has as an activity the cultivation of vines in ecological system (Figure 6) (Ecological certificate 22/162368/1458484 dated 30 September 2022). The varieties grown on the farm are Fetească Regală (on an area of 3.00 ha) and Merlot (on an area of 1.45 ha).

(**a**) (**b**)

Figure 6. Map with the grapevine plantation; (**a**) satellite image [39], (**b**) terrestrial image (first author's personal archive).

Fetească Regală (Figure 7a) is a Romanian variety of white wine grapes belonging to the category of semi-aromatic varieties, like Chardonnay. Merlot (Figure 7b) is a grape variety used to produce red wines and is cultivated in most wine-growing regions of the world. The year 2022 was a favorable year for grapevine culture, thanks to the good weather conditions resulting in productions of 8 tons/ha for the Fetească Regală variety and 6 tons/ha for the Merlot variety. To obtain wine from the Fetească Regală variety, a traditional method was used, which involves reception of the grapes, crushing and de-stemming (evacuation of the bunches), sulfiting, mustering, separation of the wine by pressing the marc, and evacuation of the pomace. For the Merlot variety, the grapes are received, crushed and de-stemmed (clusters are removed), sulfiting, mustering, maceration, fermentation on the marc until the end of the lactic fermentation (approximatively one month), and pressing the pomace and removing the residues.

a b

Figure 7. *Vitis vinifera* L. varieties: (**a**) Fetească Regală, (**b**) Merlot (first author's personal archive).

The samples were comparatively analyzed from the point of view of the macro- and microscopic characteristics and the extraction yield. The oils obtained by three extraction

methods were analyzed from the point of view of the fatty acids and total polyphenol content and of the antioxidant action.

4.2. Seed Samples

The white grapes of Fetească Regală and red grapes from the Merlot variety were harvested in the fall of 2022. After removing the bunches, the grapes were crushed, and then those from the Fetească Regală variety were left to macerate for a short time while those from the Merlot variety were left to macerate and ferment on the marc. Then, it was pressed, and the marc was removed. From the marc, the seeds were selected by sifting with sieves of different sizes and air dried at room temperature until the moisture content was below 10% [40]. After drying, the seeds were crushed into powder in a grinder for 30 s to a particle size of less than 0.5 mm. The 2 varieties of grape seeds are kept in the herbarium of the Pharmaceutical Botany Laboratory, Pharmacy Department, Faculty of Medicine and Pharmacy, University of Oradea, for further research.

4.3. Macroscopic and Microscopic Analyses

The macroscopic characters of the seeds were studied, considering the Romanian Pharmacopoeia, 10th edition [41], by observing the organoleptic characters: appearance (size, color), scent, and flavor. Microscopic control was carried out on the fresh vegetable product, included in the elderberry marrow, and sectioned with the help of a blade. Cross-sections were then cleared and stained. Staining was performed to highlight the types of tissues that make up the seed or to locate specific compounds (oil). The processing consisted of making cross-sections through the seeds, which were then pigmented with a hydroalcoholic solution of Genevez reagent (Congo red and chrysoidine). The sections were kept for 5 min, then washed several times with distilled water to remove excess dye. The Sudan III reagent was used for the histochemical localization of the fatty oil from the seeds [25]. An Optika microscope model C-B10+ (BG-Italy, 24010 Ponteranica, Italy) equipped with a OpticamB10 digital camera (BG-Italy, 24010 Ponteranica, Italy) was used to make observations and capture pictures.

4.4. Chemicals and Reagents

n-Hexane was purchased from Merck KGaA, Darmstadt, Germany. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 1,1-Diphenyl-2-picrylhydrazyl-hydrate (DPPH), 2,4,6-Tris(2-pyridyl)-S-triazine (TPTZ), Neocuproine (2.9-dimethyl-1, and 10-phenantroline) were purchased from Sigma Aldrich, St. Louis, MO, in the United States. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt 98% (ABTS) was purchased from Thermo Fisher Scientific, Waltham, MA, USA. Dimethyl sulfoxide pure was purchased from Chempur, Jana Lortza, Poland. Gallic acid 98%, Folin–Ciocalteu reagent, iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), copper (II) chloride ($CuCl_2$), and sodium carbonate (Na_2CO_3) were purchased from Carl Roth GmbH + Co. KG, Karlsruhe, Germany. Methanol was purchased from Chimreactiv SRL, Bucuresti, Romania. The lipid standards used from preparation of fatty acid methyl esters (FAMEs) were purchased from Sigma–Aldrich, St. Louis, MO, USA.

4.5. Extraction Techniques

4.5.1. Ultrasound-Assisted Extraction

For the US extraction, 30 g of grape seed powder from both varieties were used, over which 250 mL of hexane was added (Table 5). The samples were placed in an Elma Elmasonic S100H (Elma Schmidbauer GmbH, Singen, Germany) ultrasonic bath, with a power of 550 W, frequency of 37 kHz, and at a constant temperature of 30 °C, for 90 min [23]. Afterwards, the extracts were filtered under vacuum, and the solvent was removed with a rotary evaporator under vacuum at 40 °C. The obtained oil was placed in glass vials and kept at 4 °C until further determinations.

4.5.2. Cold Extraction under Stirring

In total, 30 g of grape seed powder from the 2 varieties were subjected to extraction, together with 250 mL of hexane for 90 min, at room temperature, by simply stirring on an IKAmag RCT magnetic stirrer (IKA®-Werke GmbH & Co. KG, Staufen, Germany). After filtering, the extraction solvent was evaporated under pressure, at a bath temperature of 40 °C and a rotational speed of 30 rpm, in a rotating evaporator made by Heidolph Instruments, Berlin, Germany. The obtained oil was placed in glass vials and kept at 4 °C until further determinations [36].

4.5.3. Microwave-Assisted Extraction

MW-assisted extraction was performed, according to the literature [42,43] with some modifications. The experiments were performed by using a Microwave Extraction Reactor (Betameg Invest SRL, Bucharest, Romania), with adjustable power between 0–900 W and frequency of 2450 MHz. An amount of 20 g of grape seed powder was introduced into the cartridge. An amount of 400 mL of hexane (Table 6) was introduced into the solvent vessel. The matrix-to-solvent ratios were modified in accordance with the constraints of the experimental design [19,36].

Table 6. Experimental factors for producing GSO under various handling conditions.

Sample	Extraction Technique	Process Condition
Red grape seeds Merlot (M)		
GSO_M_US	Ultrasound-Assisted Extraction	Solvent: n-hexan, 37 kHz, 30 °C, 90 min
GSO_M_stirring	Conventional Extraction	Solvent: n-hexan, 90 min, room temperature
GSO_M_MW	Microwave-Assisted Extraction	Solvent: n-hexan, 300 W, 30 s ON, 8 min OFF, 40 min, 38 °C
White grape seeds Fetească Regală (FR)		
GSO_FR_US	Ultrasound-Assisted Extraction	Solvent: n-hexan, 37 kHz, 30 °C, 90 min
GSO_FR_stirring	Conventional Extraction	Solvent: n-hexan, 90 min, room temperature
GSO_FR_MW	Microwave-Assisted Extraction	Solvent: n-hexan, 300 W, 30 s ON, 8 min OFF, 40 min, 38 °C

The extraction was performed at MW irradiation power of 300 W, and the irradiation time was 30 s, followed by a break of 8 min. The total extraction period was 40 min, with 6 successive irradiations being carried out every 8 min. The extractive solution was subjected to the process of removing the solvent in a rotary evaporator (Heidolph Instruments, Berlin, Germany) under vacuum at 40 °C. The obtained oil was placed in dark bottles and kept at 4 °C.

4.5.4. Extraction Yield and Physical Indices

After the extraction of the oil using the 3 methods, the physical characterization was carried out, determining the extraction yield (%), density as a ratio between mass (g) and volume (mL), refractive index, and color. Following each extraction, the oil yield was determined using Equation (1).

$$\text{Yield (\%)} = m_{oil}/m_{seed} \times 100 \quad (1)$$

The refractive index of GSO was determined at room temperature with the Abbe Refractometer, OPTIKA model 2WAJ (Optika SRL, Ponteranica, Italia) [36].

4.6. Chemical Characterization of Grape Seed Oil

4.6.1. Fatty Acid Determination from GSO

Total lipid extracts were trans esterified into fatty acid methyl esters (FAMEs) using the acid-catalyzed procedure and analyzed with a gas chromatograph (GC) coupled to a mass spectrometer (MS) (PerkinElmer Clarus 600 T GC-MS; PerkinElmer, Inc., Shelton, CT, USA) [44].

The system was equipped with a Supelcowax 10 capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness; Supelco Inc., Bellefonte, PA, USA), and the operating conditions were as follows: injector temperature 210 °C; helium carrier gas flow rate 0.8 mL/min; injection volume 1 µL; split ratio 1:24; oven temperature 140 °C (hold 2 min) to 220 °C at 7 °C/min (hold 23 min); electron impact ionization voltage 70 eV; trap current 100 µA; ion source temperature 150 °C; mass range 22−395 *m/z* (0.14 scans/s with an intermediate time of 0.02 s between the scans). The methylated fatty acid peak identification was based on comparison of both retention time and MS of the unknown peak to those of known standards (37 components FAME Mix, Supelco no. 47885 47885 U) and with data provided by MS database (NIST MS Search 2.0). The amount of each fatty acid was calculated as the individual peak area percentage from the total fatty acid content.

4.6.2. Functional Quality

Three metrics derived and computed from fatty acid (FA) profiles were used to assess the functional value of GSOs. Equation (2) was used to determine the ratio of FAs with hypo- and hypercholesterolemia (H/H) [45].

$$\frac{H}{H} = \frac{C18:1 + C18:2 + C18:3}{C14:0 + C16:0} \quad (2)$$

The thrombogenicity index (TI) and atherogenicity index (AI) were also determined using Equations (3) and (4) [46,47].

$$AI = \frac{C14:0 + 4(C16:0)}{\sum MUFA + \sum \omega - 3 + \sum \omega - 6} \quad (3)$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5(\sum MUFA) + 3\sum \omega - 3 + 0.5\sum \omega - 6 + \left(\frac{\sum \omega - 3}{\sum \omega - 6}\right)} \quad (4)$$

Linoleic acid is C18:2, α-linolenic acid is C18:3, myristic acid is C14:0, oleic acid is C18:1, palmitic acid is C16:0, and stearic acid is C18:0. ΣMUFA is the total of monounsaturated FAs, $\Sigma\omega - 6$ is the total of polyunsaturated fatty $\omega - 6$ acids, and $\Sigma\omega - 3$ is the total of polyunsaturated $\omega - 3$ FAs.

4.6.3. Determination of Total Phenolic Content

The total phenolic content of GSO was measured by using a modified Folin–Ciocalteu method. First, GSO was diluted 1:1 (*v*/*v*) in dimethyl sulfoxide (sample) [48]. In total, 200 µL of recently prepared Folin–Ciocalteu reagent (1:10 dilution (*v*/*v*)), 100 µL GSO, 1700 µL of distilled water, and 7.5% Na_2CO_3 solution were combined. The mix was left to sit at room temperature in the dark for two hours. The absorbance was recorded at 765 nm using the spectrophotometer (PG Instruments Ltd., Leicestershire, UK) and using gallic acid as a reference; the findings were reported in milligrams of gallic acid equivalent (GAE) per g of oil (y = 0.0236x + 0.008, R^2 = 0.9998) [49].

4.7. Antioxidant Capacity Determination of Grape Seed Oil

The antioxidant capacity of GSO was measured using four different methods. Before starting the determinations, GSO obtained by the three methods was diluted 1:1 (*v*/*v*) in dimethyl sulfoxide (sample) [50].

4.7.1. DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate) Assay

The radical DPPH scavenging ability of GSO was calculated using a technique taken from the scientific literature [49]. A quantity of 100 µL of sample was blended with 2800 µL of recently made 80 µM DPPH methanol solution, and the mixture was then incubated at room temperature for precisely 30 min in the dark. The absorbance was assessed at 517 nm, and

the radical scavenging capacity was determined using Equation (5), where A_0 represents the absorbance of blank, and A_1 represents the absorbance of the sample under investigation.

$$\% \text{ Radical Scavenging Activity} = [(A_0 - A_1)/A_0] \times 100 \quad (5)$$

A calibration curve was designed by plotting the amount of% DPPH inhibition that was scavenged against the concentration of a standard antioxidant (0.125–4 mM Trolox) (y = 5.2384x + 0.3069, R^2 = 0.9983). Outcomes were indicated in terms of µmol Trolox equivalent (TE)/g of oil.

4.7.2. FRAP (Ferric Reducing Antioxidant Power) Assay

In essence, 100 µL of sample was combined with 2000 µL of distilled water and 500 µL of the FRAP working solution, which was made up of recently prepared solutions of 20 mM $FeCl_3 \cdot 6H_2O$ solution, 10 mM 2,4,6-tripyridyl-S-triazine solution (TPTZ), and 300 mM acetate buffer, pH3.6, in ratios of 10:1:1 (*v*/*v*/*v*). This mixture was kept at room temperature and in the dark for an hour. The data were presented as µmol TE/g of oil after the absorbance was determined at 595 nm. Trolox (0.03125–0.5 mM) served as a reference solution [49], and the regression equation coefficient of the measurement for the calibration curve was R^2 = 0.9956 (y = 1.6549x + 0.3522).

4.7.3. ABTS (2,20-Azino-Bis [3-Ethylbenzothiazolin-6-Sulfonic Acid]) Assay

A technique optimized from the literature was used to assess the sample's capacity to scavenge ABTS radicals [49]. Basically, ABTS solution 7 mM and potassium persulphate solution 2.45 mM were combined, and the mixture was left in the dark for 12 h to create the $ABTS^{\bullet+}$ cation radical. After that, the obtained ABTS solution was diluted in a phosphate buffer with a pH of 6.7 so that the absorbance at 730 nm would be 0.70 ± 0.02. The absorbance was measured at 730 nm exactly 1 min after adding100 µL of sample to 2400 µL of diluted ABTS cation radical solution. The antioxidant capacity of GSO was expressed as µmol Trolox equivalent (TE)/g of oil. Using 0.6–40 µM Trolox as reference, and the calibration curve was generated (y = 1902.9x + 3.0018, R^2 = 0.9989).

4.7.4. CUPRAC (Cupric Reducing Antioxidant Capacity) Assay

The procedure involves mixing an antioxidant extract with a copper (II) chloride solution (1×10^{-2} M), an ammonium acetate aqueous buffer (pH 7), and a Neocuproine (2.9-dimethyl-1, 10-phenantroline) alcoholic solution (7.5×10^{-3} M), then determining the absorbance at 450 nm after 30 min [49]. Thus, the procedure was carried out by adding 100 µL of sample, 1 mL of Neocuproine solution, 1 mL of $CuCl_2$ solution, 1 mL of ammonium acetate buffer, and then 4.1 mL of water. The findings were given in µmol of TE per gram of oil by using Trolox as reference (0.0156–0.25 mM), and the calibration curve was generated (y = 3.826x + 0.008, R^2 = 0.9988).

4.8. Statistical Analysis

The statistical analysis was performed using one-way ANOVA and the Tuckey test via SPSS statistical package (version 25, Chicago, IL, USA). To evaluate relationships between TPC and GSO antioxidant potential, Pearson correlation coefficient (r) was used. Data are reported as mean ± SD; all determinations were made in triplicate.

5. Conclusions

According to the findings, independent of the technique of extraction, grape seeds from the two cultivars unde r study were a good source of high-quality GSOs, rich in linoleic acid, with high amounts of total phenolics and good antioxidant capacity. However, further research involving extraction without organic solvents and determining the chemical profile of compounds with antioxidant potential is needed to accurately determine the bioactive properties of the oils obtained from the varieties studied. The results offer new perspectives and research opportunities to incorporate these extracts into pharmaceuticals, different functional

foods, or cosmetic products. The option of making use of the waste in seed oil extraction seems to be extremely profitable, given the abundance of grape pomace in Romania.

Author Contributions: Conceptualization, M.A.G. and M.B.P.; Data curation, M.A.G., M.A.B. and F.V.D.; Formal analysis, D.G. and D.M.T.; Investigation, M.A.G., D.G., M.A.B., F.V.D. and M.B.P.; Methodology, D.G. and D.M.T.; Software, A.-F.R.; Supervision, D.M.T. and S.G.B.; Validation, S.G.B.; Writing—original draft, D.G., D.M.T., S.G.B., A.-F.R. and M.B.P.; Writing—review and editing, D.M.T., S.G.B. and A.-F.R. All authors have read and agreed to the published version of the manuscript.

Funding: The research has been funded by the University of Oradea, within the Grants Competition "Scientific Research of Excellence Related to Priority Areas with Capitalization through Technology Transfer: INO—TRANSFER—UO", Project No. 308/2021.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the two first authors. The literature data are in accordance with the references inserted.

Acknowledgments: This work was supported by a grant from the Ministry of Research, Innovation and Digitization, CNCS-UEFISCDI, project number PN-III-P4-PCE-2021-G750, within PNCDI in. The authors would like to thank S.C. Gentis Prest SRL for the materials used for experiments, as well as Research grant—no. 127/25.06.2021 (using microwave apparatus obtained through this project).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sargolzaei, M.; Rustioni, L.; Cola, G.; Ricciardi, V.; Bianco, P.A.; Maghradze, D.; Failla, O.; Quaglino, F.; Toffolatti, S.L.; De Lorenzis, G. Georgian Grapevine Cultivars: Ancient Biodiversity for Future Viticulture. *Front. Plant Sci.* **2021**, *12*, 630122. [CrossRef] [PubMed]
2. Antoce, A.O.; Calugaru, L.L. Evolution of grapevine surfaces in Romania after accession to European Union—period 2007–2016. *BIO Web Conf.* **2017**, *9*, 1–7. [CrossRef]
3. OIV: Organisation Internationale de la Vigne et du vin Global Economic Vitiviniculture Data. Available online: https://www.oiv.int/ (accessed on 21 March 2023).
4. Oprea, O.; Apostol, L.; Bungau, S.B.; Cioca, G.; Samuel, A.D.; Badea, M.; Gaceu, L. Researches on the chemical composition and the rheological properties of wheat and grape epicarp flour mixes. *Rev. Chim.* **2018**, *69*, 70–75. [CrossRef]
5. Badea, M.; di Modugno, F.; Floroian, L.; Tit, D.M.; Restani, P.; Bungau, S.; Iovan, C.; Badea, G.E.; Aleya, L. Electrochemical strategies for gallic acid detection: Potential for application in clinical, food or environmental analyses. *Sci. Total Environ.* **2019**, *672*, 129–140. [CrossRef]
6. Shinagawa, F.B.; de Santana, F.C.; Torres, L.R.O.; Mancini-Filho, J. Grape seed oil: A potential functional food? *Food Sci. Technol.* **2015**, *35*, 399–406. [CrossRef]
7. Lucarini, M.; Durazzo, A.; Romani, A.; Campo, M.; Lombardi-Boccia, G.; Cecchini, F. Bio-Based Compounds from Grape Seeds: A Biorefinery Approach. *Molecules* **2018**, *23*, 1888. [CrossRef]
8. Argon, Z.U.; Celenk, V.U.; Gumus, Z.P. Cold pressed grape (*Vitis vinifera*) seed oil. In *Cold Pressed Oils: Green Technology, Bioactive Compounds, Functionality, and Applications*; Academic Press: New York, NY, USA, 2020; pp. 39–52.
9. Abdel-Daim, M.M.; Abo-EL-Sooud, K.; Aleya, L.; Bungau, S.G.; Najda, A.; Saluja, R. Alleviation of Drugs and Chemicals Toxicity: Biomedical Value of Antioxidants. *Oxid. Med. Cell. Longev.* **2018**, *2018*, 6276438. [CrossRef]
10. Hornedo-Ortega, R.; González-Centeno, M.R.; Chira, K.; Jourdes, M.; Teissedre, P.-L.; Hornedo-Ortega, R.; González-Centeno, M.R.; Chira, K.; Jourdes, M.; Teissedre, P.-L. Phenolic Compounds of Grapes and Wines: Key Compounds and Implications in Sensory Perception. In *Chemistry and Biochemistry of Winemaking, Wine Stabilization and Aging*; IntechOpen: London, UK, 2020; pp. 1–26.
11. Gouot, J.C.; Smith, J.P.; Holzapfel, B.P.; Walker, A.R.; Barril, C. Grape berry flavonoids: A review of their biochemical responses to high and extreme high temperatures. *J. Exp. Bot.* **2019**, *70*, 397–423. [CrossRef]
12. Kapcsándi, V.; Hanczné Lakatos, E.; Sik, B.; Linka, L.Á.; Székelyhidi, R. Characterization of fatty acid, antioxidant, and polyphenol content of grape seed oil from different *Vitis vinifera* L. varieties. *OCL* **2021**, *28*, 1–6. [CrossRef]
13. Galili, S.; Hovav, R. Determination of Polyphenols, Flavonoids, and Antioxidant Capacity in Dry Seeds. In *Polyphenols in Plants: Isolation, Purification and Extract Preparation*; Academic Press: New York, NY, USA, 2014; pp. 305–323.
14. Folin, O.; Ciocalteu, V. On Tyrosine and Tryptophane Determinations in Proteins. *J. Biol. Chem.* **1927**, *73*, 627–650. [CrossRef]
15. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.

16. Pallag, A.; Bungau, S.G.; Tit, D.M.; Jurca, T.; Sirbu, V.; Honiges, A.; Horhogea, C. Comparative Study of Polyphenols, Flavonoids and Chlorophylls in *Equisetum arvense* L. Populations. *Rev. Chim.* **2016**, *67*, 530–533.
17. Lamuela-Raventós, R.M. Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity. In *Measurement of Antioxidant Activity and Capacity: Recent Trends and Applications*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2017; pp. 107–115.
18. Duba, K.; Fiori, L. Supercritical CO_2 extraction of grape seeds oil: Scale-up and economic analysis. *Int. J. Food Sci. Technol.* **2019**, *54*, 1306–1312. [CrossRef]
19. Krishnaswamy, K.; Orsat, V.; Gariépy, Y.; Thangavel, K. Optimization of Microwave-Assisted Extraction of Phenolic Antioxidants from Grape Seeds (*Vitis vinifera*). *Food Bioprocess Technol.* **2013**, *6*, 441–455. [CrossRef]
20. Makarova, N.V.; Valiulina, D.F.; Eremeeva, N.B. Comparative studies of extraction methods of biologically-active substances with antioxidant properties from grape seed (*Vitis vinifera* L.). *Proc. Univ. Appl. Chem. Biotechnol.* **2020**, *10*, 140–148. [CrossRef]
21. Cristea, O.; Catana, L.; Bracacescu, C.; Baltatu, C.V.; Vladutoiu, L.; Grigore, I.; Ungureanu, N. Processing methods of wine by-products to obtain grape seed oil and grape seed flour as functional ingredients for food fortification. In Proceedings of the Actual Tasks on Agricultural Engineering, Zagreb, Croatia, 2–4 March 2021; pp. 445–453.
22. Aziz, R.; Popescu, M.L.; Mihele, D. Contributions to the Pharmacognostical Study on Grapes Hamburg Cultivar. *Farmacia* **2008**, *56*, 571–576.
23. Dimić, I.; Teslić, N.; Putnik, P.; Kovačević, D.B.; Zeković, Z.; Šojić, B.; Mrkonjić, Ž.; Čolović, D.; Montesano, D.; Pavlić, B. Innovative and Conventional Valorizations of Grape Seeds from Winery By-Products as Sustainable Source of Lipophilic Antioxidants. *Antioxidants* **2020**, *9*, 568. [CrossRef]
24. Gitea, M.A.; Bungau, S.G.; Gitea, D.; Pasca, B.M.; Purza, A.L.; Radu, A.-F. Evaluation of the Phytochemistry-Therapeutic Activity Relationship for Grape Seeds Oil. *Life* **2023**, *13*, 178. [CrossRef]
25. Nowshehri, J.A.; Bhat, Z.A.; Shah, M.Y. Pharmacognostic Standardisation and Phytochemical Evaluation on the Seeds of Two *Vitis Vinefera* L. Varieties Grown in Kashmir Valley, India. *Pharmacogn. J.* **2016**, *8*, 465–470. [CrossRef]
26. Özcan, M.M.; Al Juhaimi, F.; Gülcü, M.; Uslu, N. Geçgel Determination of Bioactive Compounds and Mineral Contents of Seedless Parts and Seeds of Grapes. *S. Afr. J. Enol. Vitic.* **2017**, *38*, 212–220.
27. Tangolar, S.G.; Özoğul, Y.; Tangolar, S.; Torun, A. Evaluation of fatty acid profiles and mineral content of grape seed oil of some grape genotypes. *Int. J. Food Sci. Nutr.* **2009**, *60*, 32–39. [CrossRef] [PubMed]
28. Al Juhaimi, F.; Geçgel; Gülcü, M.; Hamurcu, M.; Özcan, M.M. Bioactive Properties, Fatty Acid Composition and Mineral Contents of Grape Seed and Oils. *S. Afr. J. Enol. Vitic.* **2017**, *38*, 103–108. [CrossRef]
29. Garavaglia, J.; Markoski, M.M.; Oliveira, A.; Marcadenti, A. Grape Seed Oil Compounds: Biological and Chemical Actions for Health. *Nutr. Metab. Insights* **2016**, *9*, 59–64. [CrossRef] [PubMed]
30. Rombaut, N.; Savoire, R.; Thomasset, B.; Castello, J.; Van Hecke, E.; Lanoisellé, J.L. Optimization of oil yield and oil total phenolic content during grape seed cold screw pressing. *Ind. Crops. Prod.* **2015**, *63*, 26–33. [CrossRef]
31. Li, H.; Fu, X.; Deng, G.; David, A.; Huang, L. Extraction of oil from grape seeds (*Vitis vinifera* L.) using recyclable CO_2-expanded ethanol. *Chem. Eng. Process.-Process Intensif.* **2020**, *157*, 108147. [CrossRef]
32. Nehdi, I.A. Cupressus sempervirens var. horizentalis seed oil: Chemical composition, physicochemical characteristics, and utilizations. *Ind. Crops Prod.* **2013**, *41*, 381–385. [CrossRef]
33. Konuskan, D.B.; Kamiloglu, O.; Demirkeser, O. Fatty Acid Composition, Total Phenolic Content and Antioxidant Activity of Grape Seed Oils Obtained by Cold- Pressed and Solvent Extraction. *Indian J. Pharm. Educ. Res.* **2019**, *53*, 144–150. [CrossRef]
34. Hashempour-Baltork, F.; Torbati, M.; Azadmard-Damirchi, S.; Savage, G.P. Chemical, Rheological and Nutritional Characteristics of Sesame and Olive Oils Blended with Linseed Oil. *Adv. Pharm. Bull.* **2018**, *8*, 107–113. [CrossRef]
35. Pinto, R.H.H.; Menezes, E.G.O.; Freitas, L.C.; Andrade, E.H.d.A.; Ribeiro-Costa, R.M.; Silva, J.O.C., Jr.; Carvalho, R.N., Jr. Supercritical CO_2 extraction of uxi (Endopleura uchi) oil: Global yield isotherms, fatty acid profile, functional quality and thermal stability. *J. Supercrit. Fluids* **2020**, *165*, 104932. [CrossRef]
36. De Souza, R.d.C.; Souza Machado, B.A.; de Abreu Barreto, G.; Leal, I.L.; dos Anjos, J.P.; Umsza-Guez, M.A. Effect of Experimental Parameters on the Extraction of Grape Seed Oil Obtained by Low Pressure and Supercritical Fluid Extraction. *Molecules* **2020**, *25*, 1634. [CrossRef]
37. Mollica, A.; Scioli, G.; Valle, A.D.; Cichelli, A.; Novellino, E.; Bauer, M.; Kamysz, W.; Llorent-Martínez, E.J.; De Córdova, M.L.F.; Castillo-López, R.; et al. Phenolic Analysis and In Vitro Biological Activity of Red Wine, Pomace and Grape Seeds Oil Derived from *Vitis Vinifera* L. cv. Montepulciano d'Abruzzo. *Antioxidants* **2021**, *10*, 1704. [CrossRef]
38. Herrero, M.; Castro-Puyana, M.; Mendiola, J.A.; Ibañez, E. Compressed fluids for the extraction of bioactive compounds. *TrAC Trends Anal. Chem.* **2013**, *43*, 67–83. [CrossRef]
39. Google Map with the Grapevine Plantation. Available online: https://www.google.com/maps/place/47°16$'$10.6%22N+22°08$'$04.2%22E/@47.26962,22.1327663,578m/data=!3m2!1e3!4b1!4m13!1m8!3m7!1s0x4747ca07a65c2231:0x4fcaa48c15f119dc!2sSîniob+417192!3b1!8m2!3d47.2638987!4d22.128266!16s%2Fg%2F1q6j717xq!3m3!8m2!3d47.26962!4d22.1 (accessed on 8 April 2023).
40. Dabetic, N.M.; Todorovic, V.M.; Djuricic, I.D.; Stankovic, J.A.A.; Basic, Z.N.; Vujovic, D.S.; Sobajic, S.S. Grape Seed Oil Characterization: A Novel Approach for Oil Quality Assessment. *Eur. J. Lipid Sci. Technol.* **2020**, *122*, 1900447. [CrossRef]

41. Pharmacognostic determinations, Macroscopic and microscopic control of plant products. In *Romanian Pharmacopoeia*; Bucharest Medical Publishing House: Bucharest, Romania, 1993; pp. 1051–1053.
42. Hong, N.; Yaylayan, V.A.; Vijaya Raghavan, G.S.; Paré, J.R.J.; Bélanger, J.M.R. Microwave-assisted Extraction of Phenolic Compounds from Grape Seed. *Nat. Prod. Lett.* **2001**, *15*, 197–204. [CrossRef]
43. Bandici, L.; Teusdea, A.C.; Soproni, V.D.; Hathazi, F.I.; Arion, M.N.; Molnar, C.O.; Vicas, S.I. The Influence of Microwave Treatments on Bioactive Compounds and Antioxidant Capacity of *Mentha piperita* L. *Materials* **2022**, *15*, 7789. [CrossRef]
44. Dulf, F.V.; Vodnar, D.C.; Toşa, M.I.; Dulf, E.H. Simultaneous enrichment of grape pomace with γ-linolenic acid and carotenoids by solid-state fermentation with Zygomycetes fungi and antioxidant potential of the bioprocessed substrates. *Food Chem.* **2020**, *310*, 125927. [CrossRef]
45. Santos-Silva, J.; Bessa, R.J.B.; Santos-Silva, F. Effect of genotype, feeding system and slaughter weight on the quality of light lambs: II. Fatty acid composition of meat. *Livest. Prod. Sci.* **2002**, *77*, 187–194. [CrossRef]
46. Cunha, V.M.B.; da Silva, M.P.; de Sousa, S.H.B.; Bezerra, P.D.N.; Menezes, E.G.O.; da Silva, N.J.N.; Banna, D.A.D.d.S.; Araújo, M.E.; de Carvalho, R.N., Jr. Bacaba-de-leque (Oenocarpus distichus Mart.) oil extraction using supercritical CO_2 and bioactive compounds determination in the residual pulp. *J. Supercrit. Fluids* **2019**, *144*, 81–90. [CrossRef]
47. Ulbricht, T.L.V.; Southgate, D.A.T. Coronary heart disease: Seven dietary factors. *Lancet* **1991**, *338*, 985–992. [CrossRef]
48. Eroglu, E.; Girgin, S.N. A unique phenolic extraction method from olive oil macerate of *Hypericum perforatum* using DMSO: Assessment of in vitro anticancer activity, LC-MS/MS profile, total phenolic content and antioxidant capacity. *S. Afr. J. Bot.* **2021**, *139*, 6–11. [CrossRef]
49. Schlesier, K.; Harwat, M.; Böhm, V.; Bitsch, R. Assessment of antioxidant activity by using different in vitro methods. *Free Radic. Res.* **2002**, *36*, 177–187. [CrossRef] [PubMed]
50. Fratianni, F.; D'Acierno, A.; Ombra, M.N.; Amato, G.; De Feo, V.; Ayala-Zavala, J.F.; Coppola, R.; Nazzaro, F. Fatty Acid Composition, Antioxidant, and in vitro Anti-inflammatory Activity of Five Cold-Pressed *Prunus* Seed Oils, and Their Anti-biofilm Effect Against Pathogenic Bacteria. *Front. Nutr.* **2021**, *8*, 775751. [CrossRef] [PubMed]