

## Article

# Valorization of Winery Waste: Prokupac Grape Seed as a Source of Nutritionally Valuable Oil

Jelena Milanović<sup>1</sup>, Marko Malićanin<sup>1</sup>, Vesna Rakić<sup>2</sup>, Nenad Jevremović<sup>3</sup>, Ivana Karabegović<sup>4</sup>   
and Bojana Danilović<sup>4,\*</sup> 

<sup>1</sup> Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Serbia; milanovic.jelena@ni.ac.rs (J.M.); malicanin.marko@ni.ac.rs (M.M.)

<sup>2</sup> Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11000 Belgrade, Serbia; vesna.rakic@agrif.bg.ac.rs

<sup>3</sup> Rubin A.D., Nade Marković 57, 37000 Kruševac, Serbia; nenad.jevremovic@rubin.rs

<sup>4</sup> Faculty of Technology, University of Niš, Bulevar Oslobođenja 124, 16000 Leskovac, Serbia; ivana.karabegovic@tf.ni.ac.rs

\* Correspondence: bojana.danilovic@junis.ni.ac.rs

**Abstract:** Valorization of winery waste is a promising solution for waste management with additional benefit of bioactive compounds isolation. The aim of this study was to analyze the nutritional and bioactive potential of oil derived from Serbian autochthonous grape seed variety Prokupac and to compare it with known international grape varieties. Additionally, two extraction methods (ultrasound assisted extraction and cold pressing) were used in order to determine their influence on physico-chemical and nutritive characteristics of grape seed oil. Different methods, including determination of physicochemical properties, content of bioactive compounds, oxidative stability and antioxidative capacity were used for the characterization of grape seed oils. Results indicated that the higher yield was obtained by ultrasound assisted extraction compared to cold pressing. The application of ultrasound assisted extraction resulted in increased  $\alpha$ -tocopherol content and better antioxidant capacity and oxidative stability of oil. Significant differences in bioactive composition were observed for Prokupac seed oil compared to the other grape varieties. Oil extracted from Prokupac grape seed had the lowest content of polyunsaturated and the highest content of saturated fatty acids, the highest content of  $\alpha$ -tocopherol and consequently, the best antioxidative capacity. Prokupac grape seed has a great potential for isolation of nutritive and bioactive valuable oil.

**Keywords:** grape seed oil; winery waste; Prokupac



**Citation:** Milanović, J.; Malićanin, M.; Rakić, V.; Jevremović, N.; Karabegović, I.; Danilović, B. Valorization of Winery Waste: Prokupac Grape Seed as a Source of Nutritionally Valuable Oil. *Agronomy* **2021**, *11*, 1864. <https://doi.org/10.3390/agronomy11091864>

Academic Editors: Fogarassy Csaba, Csaba Gyuricza and Richard Cruse

Received: 25 August 2021

Accepted: 13 September 2021

Published: 16 September 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Current trends in food production are focused on the development of sustainable and more efficient strategies for industrial and agricultural waste valorization. Although it represents environmental and economic problems, agro-industrial wastes possess an enormous potential as a raw material for the production of high-added value products [1]. The wine industry, like some others, is known to generate a significant volume of solid waste (20% of processed grape weight consisted mainly of seeds, skins, pulp) rich in nutritionally valuable and biologically active compounds. Special attention is given to grape seed rich in oil, protein, antioxidants, phenolic compounds, non-phenolic antioxidants (tocopherols and beta-carotene), fibers, mineral substances and tannins [2]. The content and composition of grape seed oil is influenced by many factors, such as grape variety, method of isolation, degree of maturity, climate and growing conditions [2,3]. According to the literature data, seeds of different grape varieties contain from 6% to 20% of oil [4]. Additionally, some authors reported higher content of oil for white varieties compared to the red ones [5]. Analysis of fourteen vegetable oils indicated that safflower oil and grape seed oil are the richest source of linoleic acid (79% and 74.7%, respectively) and

polyunsaturated fatty acids (PFA) [6]. In addition to healthy and nutritionally valuable fatty acids composition, grape seed oil represents good sources of vitamin E and polyphenolic compounds which, additionally, provide the high stability of oil [7]. Evaluation of 31 grape varieties for phenolic compounds, antioxidant and antiproliferative activities, indicated that *V. vinifera* seed has the greatest potential as a source natural antioxidative compounds [8].

The presence of antioxidants (polyphenols, tocopherols and tocotrienols) in vegetable oils is essential for stability of PFA and prevention of oxidative spoilage of oil [9]. The quality and oxidative stability of oil may be influenced by the phase of the fermentation in which the seeds was collected [10]. In addition, one of the factors which may influence the chemical characteristics of oil is the seed drying method [3,11]. Pardo and co-workers reported that drying with hot air ensured better physicochemical properties, higher total phenolic content (TPC) and higher stability of grape seed oil compared to unheated air-drying [3].

The most common and widespread methods of vegetable oils isolation include cold pressing by hydraulic or screw presses [12] and solvent extraction. In addition, it has been proven that the use of ultrasound can increase the extraction efficiency, by the action of acoustic cavitation which can destroy the cell wall and facilitate the penetration of solvents into plant material and the release of intracellular contents [12–14]. The mechanical effects caused by ultrasound can enhance solvent utilization during extraction by increasing the contact area between the solvent and the desired component. The main advantages of ultrasound application are the reduction of extraction time and reduction of solvent consumption [15]. In addition to these advantages, ultrasonic extraction can be performed at lower temperatures compared to conventional methods and, thus, thermal damage to the extract and loss of bioactive components can be avoided.

According to the Statistical Office of the Republic of Serbia in 2018, the production of grapes in Serbia was about 150,000 tons, with clear trend of increasing the production of autochthonous grape varieties, especially Prokupac, as one of the most popular autochthonous grape variety in Serbia. This reflects to the future increase of already significant amount of grape pomace which arise from Prokupac wine production. Hence, in order to assess a full potential of this grape variety, except the wine making process, the valorization of by-products is indispensable. Bearing in mind that the grape variety has a significant influence to the yield, composition, nutritional value and bioactive constituents of oil, the aim of this work was to investigate and characterize the Prokupac grape seed oil obtained by novel extraction method and compare with grape seed oils from international grape varieties.

## 2. Materials and Methods

### 2.1. Materials

Seeds of autochthonous Serbian grape variety Prokupac and four international red grape varieties (Cabernet Sauvignon, Merlot, Pinot Noir, Gamay) cultivated in vineyard of vine producer “Rubin” (Krusevac, Serbia) harvested in 2019, were used in this study. Seeds were separated during the fermentation process using a special valve on the fermentation vessel. Cleaned seeds were dried on the ambient air to moisture content below 10%. Dry seeds were ground with horizontal coffee grinder (Elektron, Dublje) and sifted through standard laboratory sieves with 0.3 mm and 0.5 mm pore size prior to ultrasound extraction. The fraction with an average particle diameter of 0.4 mm collected between sieves, was used for the experiments. Synthetic air was purchased from Messer Technogas A.D., (Novi Sad, Serbia), n-Hexane, methanol, ethanol, boron trifluoride, sodium hydroxide, potassium hydroxide, sodium thiosulphate, sodium carbonate and hydrogen chloride were purchased from Merck, (Darmstadt, Germany). Gallic acid and p-anisidine were purchased from Alfa Aesar (Thermo Fisher GmbH, Kandel, Germany) while  $\alpha$ -tocopherol was purchased from Acros Organics (Thermo Fisher GmbH, Kandel, Germany).

## 2.2. Ultrasound Assisted Extraction (UAE)

Ground grape seeds ( $50 \pm 0.001$  g) and 100 mL of *n*-hexane were put in an Erlenmeyer flask covered with aluminum foil and exposed to ultrasound for 90 min in an ultrasonic bath (Bandelin Sonorex, Berlin, Germany, volume 3 L, 35 kHz frequency, 150 W power). The extraction conditions were selected according to our preliminary experiment (unpublished data of extraction process optimization using response surface methodology—RSM). The temperature was controlled and maintained at 30 °C by water circulating from a thermostatic bath by means of a pump. Solid phase was separated by decanting and washed with *n*-hexane. The solvent was evaporated on a rotary evaporator (Senco, Shanghai, China) at 37 °C, pressure of 0.8 bar using 150 rotations per minute [16].

## 2.3. Cold Pressed Extraction (CP)

Extraction method by cold pressing was performed using hydraulic press (Hanaro, Daejeon, Korea, model A). Native (unground) dried seeds (moisture from 8 to 10%) were processed under pressure of 600 bar, at temperature of cylinder set at 50 °C. Temperature of extracted oil was 30 °C.

## 2.4. Total Oil Content—Soxhlet Method (SE)

Soxhlet method was used to determine total oil content according to standard method SRPS EN ISO:2011 [17]. Extraction of ground grape seed was conducted at 80 °C for 6 h. Solvent removing was performed on rotary vacuum evaporator (“Senco”, at 50 °C, 0.8 bar and 150 r min<sup>-1</sup>) to the constant weight.

## 2.5. Fatty Acids Composition

Standard Gas chromatographic method was applied to analyze fatty acids composition of the oils. Transesterification of triglycerides extracted from oils was done using a solution of BF<sub>3</sub>, (12 % *v/v*) in methanol, following IUPAC method 2.301 [18]. Quantitative GC analysis was performed on a GC 5160 gas chromatograph (Carlo Erba, Milano, Italy), capillary column (30 m × 0.25 mm ID × 0.25 μm film) ZB-5 ms (Phenomenex, Santa Clara, CA, USA). The chromatographic conditions were: 1 μL splitless injection at 100 °C, 3 min isothermal time, a ramp of 10 °C min<sup>-1</sup> to 220 °C, 20 min isothermal time and a second ramp of 40 °C min<sup>-1</sup> to 300 °C. The injector temperature was 270 °C and the splitless time was 60 s. Flow rate of carrier gas (helium) was 1.5 mL min<sup>-1</sup> and detector temperature (flame ionization detector) was set at 300 °C. The content (%) of fatty acids in the samples were determined on the basis of area percent report generated by software, i.e., the area of each peak as a percentage of the total area of all peaks in the run.

## 2.6. Total Phenolic Content (TPC)

For determination of phenolic compounds oil samples ( $1 \pm 0.001$  g) were extracted by diluted methanol (90:10), for 5 min, on the ultrasound bath [19]. Quantitatively transferred extracts were centrifuged at 4000 r min<sup>-1</sup> for 10 min (Focus Instruments, Hong Kong, China). All the methanolic extracts were combined and concentrated until dryness. Dry residues were dissolved with 2.5 mL diluted methanol (90:10) and kept on -20 °C until analysis. For spectrophotometric determination, 0.5 mL of the oil sample was mixed with Folin–Ciocalteu reagent according to procedure described in the literature [20]. The absorbance was recorded at 730 nm. Quantification was carried out using a standard curve and results were expressed as milligrams of gallic acid equivalents per 100 g of oil (mg GAE 100 g<sup>-1</sup>).

## 2.7. α-Tocopherol Content

HPLC method was used to determine α-tocopherol level, according to the procedure conducted by Dabbou et al. [21]. Oil sample (0.0100–0.0200 g) was prepared for analysis by dissolving in 2-propanole (1 mL). The analysis was performed at room temperature on a Bischoff HPLC liquid chromatograph (Bischoff, Leonberg, Germany) equipped with a

Rheodyne 7725i sample injector with a loop of 20  $\mu\text{L}$ , a compact pump model 2250, with a UV/VIS Lambda 1010 as the detection system, an in-line degasser (Bischoff, DG 1410) and with a Control and Data Interface LC-CaDI 22-14 (with McDAcq32-Control software Bischoff).  $\alpha$ -tocopherol was separated on a C18 reversed-phase column (250 mm  $\times$  4.6 mm, 120A, 5  $\mu\text{m}$ , ProntoSil, Bischoff). Isocratic elution with methanol-water (90:10 %, *v/v*) at a flow of 1 mL  $\text{min}^{-1}$  was applied.  $\alpha$ -tocopherol was monitored at 280 nm, at 19.25 min retention time.

### 2.8. Oxidative Stability

Oxidative stability of the oils was determined by differential scanning calorimetry (DSC) and Rancimat measurement.

The DSC analysis was conducted according to the ASTM standard E2009-08 procedure [22]. Onset point of the exothermic DSC signal, which occurred during the controlled heating of the oil sample, was used for oxidative stability assessment. Measurements were performed on differential scanning calorimeter coupled with thermogravimetry (DSC/TG111, Setaram, Caluire-et-Cuire, France), under atmospheric pressure. The Setsoft software from Setaram was used to collect data and for the determination of temperatures from DSC curves. The apparatus was calibrated with high-purity indium. The experiments were performed under a flow of synthetic air (22% of  $\text{O}_2$  and 78% of  $\text{N}_2$ ) at a flow of 25 L  $\text{min}^{-1}$ . Samples of oils were heated from 20 to 200  $^\circ\text{C}$  in an open quartz crucible at a linear heating rate of  $\beta = 10$   $^\circ\text{C min}^{-1}$ ; an empty quartz crucible was used as a reference material.

The resistance of the oil to oxidation was determined by measuring the induction period of oxidation on a Metrohm Rancimat 743 apparatus by the standard method [23]. A total of 2.5 g of oil samples were measured in the reaction vessels and test was performed at temperature of 120  $^\circ\text{C}$  and air flow was set at 20 L  $\text{h}^{-1}$ .

### 2.9. Antioxidant Capacity

Determination of the ability to neutralize 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was determined using the spectrophotometric method [24]. In order to determine the  $\text{EC}_{50}$  value, which represented the amount of oil that reduces the initial concentration of DPPH radicals by 50%, three concentrations of oil solution (25, 50 and 75 mg of oil in 1 mL of toluene) were prepared. Then, 3.9 mL of a  $10^{-4}$  M solution of DPPH radical in toluene was added to each sample and the absorbance of the solution was measured relative to the blank (pure toluene) at a wavelength of 515 nm after 30 min of mixing. DPPH concentration was plotted versus the amount of oil and the  $\text{EC}_{50}$  value was expressed in mg of oil per mg of DPPH radical.

### 2.10. Physicochemical Analysis

Peroxide, saponification and anisidine value were determined according to methodology recommended by AOCS Recommended Practice Cd 8-53, Cd 3-25 and Cd 18-90, respectively [25]. Iodine value was calculated from previously obtained data on fatty acid composition (AOCS method Cd 1c-85) [25], using the following formula:

$$\text{IV} = [(\% \text{C18:1} + \% \text{trans C18:1}) \times 0.86] + [(\% \text{C18:2} + \% \text{trans C18:2}) \times 0.86] + [(\% \text{C18:3} + \% \text{trans C18:1}) \times 0.86] \quad (1)$$

Relative density and acidity of oils was determined using standard methods, SRPS EN ISO 6883:2017 [26] and SRPS EN ISO 660:2011 [27], respectively.

### 2.11. Statistical Analysis

All experimental data from extraction procedures and chemical analysis were done in triplicate and the results were expressed as the average value with standard deviation. Statistical software (IBM SPSS Statistics 20, Chicago, IL, USA) was used for ANOVA analysis followed by Tukey post hoc test to detect the differences between the mean values at a 95% confidence level ( $p \leq 0.05$ ).

### 3. Results

Characterization of grape seed oils extracted from different grape varieties included determination of physicochemical properties (relative density, smoking point, saponification value, iodine value, acid value), content of bioactive compounds (fatty acids, total phenolic compounds,  $\alpha$ -tocopherol) oxidative stability and antioxidative capacity. The values of the determined physicochemical parameters of the oils are presented in Table 1.

**Table 1.** Physicochemical parameters of the analyzed grape seed oils.

Parameter	Extraction Method	Grape Variety				
		Pinot Noir	Gamay	Prokupac	Cabernet Sauvignon	Merlot
Relative density at 20 °C/water at 20 °C	CP	0.915 ± 0.00 <sup>Ba</sup>	0.920 <sup>Cb</sup> ± 0.00	0.918 <sup>BCb</sup> ± 0.00	0.919 ± 0.00 <sup>Cb</sup>	0.911 ± 0.00 <sup>Aa</sup>
	UAE	0.915 ± 0.00 <sup>Ca</sup>	0.912 ± 0.00 <sup>ABa</sup>	0.913 ± 0.00 <sup>BCa</sup>	0.910 ± 0.00 <sup>Aa</sup>	0.912 ± 0.00 <sup>ABa</sup>
Smoking point, °C	CP	214.13 ± 0.35 <sup>Bb</sup>	216.10 ± 0.00 <sup>Db</sup>	213.20 ± 0.20 <sup>Ab</sup>	216.13 ± 0.06 <sup>Db</sup>	215.07 ± 0.06 <sup>Cb</sup>
	UAE	210.17 ± 0.06 <sup>Aa</sup>	211.13 ± 0.06 <sup>Ba</sup>	212.17 ± 0.12 <sup>Ca</sup>	210.23 ± 0.06 <sup>Aa</sup>	210.20 ± 0.10 <sup>Aa</sup>
Saponification value, mg KOH g <sup>-1</sup>	CP	190.67 ± 1.15 <sup>Ba</sup>	185.00 ± 2.00 <sup>Aa</sup>	185.00 ± 0.00 <sup>Aa</sup>	187.33 ± 0.58 <sup>Aa</sup>	191.00 ± 0.00 <sup>Ba</sup>
	UAE	195.33 ± 0.58 <sup>Db</sup>	187.00 ± 1.00 <sup>Aa</sup>	191.67 ± 1.3 <sup>BCb</sup>	191.00 ± 1.00 <sup>Bb</sup>	194.00 ± 0.0 <sup>CDb</sup>
Iodine value, g 100 g <sup>-1</sup>	CP	135.00 ± 0.00 <sup>Bb</sup>	128.33 ± 0.58 <sup>Aa</sup>	138.00 ± 2.00 <sup>Cb</sup>	134.00 ± 0.00 <sup>Bb</sup>	130.33 ± 0.58 <sup>Aa</sup>
	UAE	133.00 ± 0.00 <sup>Ba</sup>	132.00 ± 0.00 <sup>Bb</sup>	134.67 ± 0.58 <sup>Ca</sup>	130.33 ± 0.58 <sup>Aa</sup>	134.67 ± 0.58 <sup>Cb</sup>
Acid value, mg KOH g <sup>-1</sup>	CP	0.653 ± 0.01 <sup>Ea</sup>	0.573 ± 0.01 <sup>Ca</sup>	0.613 ± 0.01 <sup>Da</sup>	0.547 ± 0.01 <sup>Ba</sup>	0.520 ± 0.01 <sup>Aa</sup>
	UAE	0.710 ± 0.01 <sup>Bb</sup>	0.813 ± 0.01 <sup>Db</sup>	0.677 ± 0.01 <sup>Ab</sup>	0.757 ± 0.01 <sup>Cb</sup>	0.717 ± 0.01 <sup>Bb</sup>

Data are expressed as a mean value ± SD ( $n = 3$ ). Different letters indicate the significant difference ( $p < 0.05$ ) within column (lower case letters) and within rows (uppercase letters). CP-cold pressed oil; UAE-ultrasound assisted extraction.

The relative density of all tested samples was slightly lower than the limit (0.920) prescribed by the Codex Alimentarius [28] and national regulation [29]. On the other hand, the results were in accordance with the literature data 0.908–0.937 [4,30–32]. Smoking point is related to the content of free fatty acids in the oil, because fatty acids have a higher vapor pressure than triacylglycerols. Oils with a higher content of free fatty acids or shorter chain fatty acids have lower values of the smoking point [30]. The values of the smoking point determined in this paper, except for the oil from varieties Cabernet sauvignon and Gamay, were slightly lower than the value of 216 °C reported in the literature [3]. Statistically significant differences in relation to the oil extraction method and grape variety were found. Higher values of smoking point in cold-pressed oils compared to oils extracted by ultrasound assisted method were in accordance with the acid values. Results obtained for saponification value (from 185 to 195.33) indicated that some oils, in terms of this parameter, were not within the prescribed limits, 188–194 [28,29], but within the scope of values reported in the literature data [4,29]. Oils obtained by UAE from seeds of all varieties had a higher saponification value compared to cold-pressed oils. Iodine values were in a very narrow range, from 128.33 to 135 and within the limits defined by the Codex Alimentarius standard [30] and national regulation [29] (from 128 to 150).

The hydrolysis of triacylglycerols produces free fatty acids which can affect the quality of oil. Acid number is an important indicator of oil quality. The values of the acid number for all analyzed grape seed oils were in the range from 0.520 to 0.813 mg KOH g<sup>-1</sup> and which is far below the maximum allowed limit [28,29] for cold pressed, virgin and crude oil (4 mg KOH g<sup>-1</sup>). That indicated that the seeds were fresh and well preserved until the moment of oil isolation.

#### 3.1. Bioactive Compounds of Oils

Table 2 presents yields and content of bioactive compounds of oils extracted from different varieties of grape, by cold pressed method and ultrasound assisted method. It is well known that cold pressed method yields lower oil quantity than other extraction methods.

**Table 2.** Yield and fatty acid composition of analyzed grape seed oils.

Content, %	Extraction Method	Grape Variety				
		Pinot Noir	Gamay	Prokupac	Cabernet Sauvignon	Merlot
Oil yield	CP	11.48 ± 0.11 <sup>Ca</sup>	11.51 ± 0.18 <sup>Ca</sup>	8.78 ± 0.16 <sup>Aa</sup>	10.29 ± 0.09 <sup>Ba</sup>	9.56 ± 0.14 <sup>Aa</sup>
	UAE	12.84 ± 0.21 <sup>Cb</sup>	13.01 ± 0.15 <sup>Cb</sup>	10.06 ± 0.12 <sup>Ab</sup>	11.69 ± 0.05 <sup>Bb</sup>	11.45 ± 0.23 <sup>Bb</sup>
	SE	15.22 ± 0.04 <sup>Dc</sup>	14.25 ± 0.03 <sup>Cc</sup>	11.84 ± 0.05 <sup>Ac</sup>	15.77 ± 0.04 <sup>Ec</sup>	12.84 ± 0.03 <sup>Bc</sup>
Palmitic acid	CP	6.86 ± 0.01 <sup>Aa</sup>	6.76 ± 0.17 <sup>ABa</sup>	7.08 ± 0.18 <sup>ABCa</sup>	7.39 ± 0.03 <sup>Ca</sup>	7.22 ± 0.17 <sup>BCa</sup>
	UAE	7.27 ± 0.02 <sup>Bb</sup>	6.85 ± 0.02 <sup>Aa</sup>	7.75 ± 0.06 <sup>Cb</sup>	7.59 ± 0.17 <sup>Ca</sup>	7.59 ± 0.02 <sup>Cb</sup>
Stearic acid	CP	3.53 ± 0.01 <sup>Aa</sup>	3.59 ± 0.00 <sup>Bb</sup>	4.23 ± 0.02 <sup>Ea</sup>	4.09 ± 0.00 <sup>Da</sup>	3.70 ± 0.01 <sup>Ca</sup>
	UAE	3.52 ± 0.01 <sup>Ba</sup>	3.28 ± 0.01 <sup>Aa</sup>	4.37 ± 0.01 <sup>Eb</sup>	4.29 ± 0.00 <sup>Db</sup>	3.76 ± 0.03 <sup>Cb</sup>
Oleic acid	CP	16.02 ± 0.03 <sup>Cb</sup>	16.32 ± 0.02 <sup>Da</sup>	16.59 ± 0.04 <sup>Eb</sup>	13.84 ± 0.00 <sup>Ab</sup>	14.36 ± 0.02 <sup>Bb</sup>
	UAE	15.69 ± 0.14 <sup>Ba</sup>	16.85 ± 0.03 <sup>Db</sup>	16.28 ± 0.09 <sup>Ca</sup>	13.58 ± 0.04 <sup>Aa</sup>	13.49 ± 0.04 <sup>Aa</sup>
Linoleic acid	CP	73.05 ± 0.07 <sup>Ca</sup>	72.57 ± 0.09 <sup>Ba</sup>	71.54 ± 0.11 <sup>Ab</sup>	74.17 ± 0.04 <sup>Db</sup>	74.15 ± 0.13 <sup>Da</sup>
	UAE	73.05 ± 0.1 <sup>Ca</sup>	72.53 ± 0.01 <sup>Ba</sup>	70.83 ± 0.05 <sup>Aa</sup>	73.92 ± 0.08 <sup>Da</sup>	74.66 ± 0.07 <sup>Eb</sup>
Linolenic acid	CP	0.28 ± 0.00 <sup>Aa</sup>	0.29 ± 0.01 <sup>Aa</sup>	0.31 ± 0.00 <sup>Aa</sup>	0.29 ± 0.02 <sup>Aa</sup>	0.31 ± 0.00 <sup>Aa</sup>
	UAE	0.26 ± 0.00 <sup>Aa</sup>	0.28 ± 0.02 <sup>Aa</sup>	0.36 ± 0.11 <sup>Aa</sup>	0.31 ± 0.01 <sup>Aa</sup>	0.29 ± 0.01 <sup>Aa</sup>
Arachidic acid	CP	0.11 ± 0.01 <sup>ABb</sup>	0.13 ± 0.0 <sup>BCb</sup>	0.11 ± 0.01 <sup>ABCa</sup>	0.10 ± 0.02 <sup>Aa</sup>	0.14 ± 0.00 <sup>Cb</sup>
	UAE	0.08 ± 0.00 <sup>Aa</sup>	0.08 ± 0.00 <sup>Aa</sup>	0.20 ± 0.02 <sup>Bb</sup>	0.17 ± 0.02 <sup>Bb</sup>	0.10 ± 0.02 <sup>Aa</sup>
Behenic acid	CP	0.15 ± 0.01 <sup>Aa</sup>	0.14 ± 0.04 <sup>Aa</sup>	0.14 ± 0.02 <sup>Aa</sup>	0.12 ± 0.01 <sup>Aa</sup>	0.13 ± 0.03 <sup>Aa</sup>
	UAE	0.14 ± 0.00 <sup>Aa</sup>	0.12 ± 0.01 <sup>Aa</sup>	0.21 ± 0.08 <sup>Aa</sup>	0.14 ± 0.00 <sup>Aa</sup>	0.11 ± 0.01 <sup>Aa</sup>
SFA	CP	10.65 ± 0.13 <sup>Aa</sup>	10.82 ± 0.08 <sup>ACa</sup>	11.56 ± 0.13 <sup>Ba</sup>	11.7 ± 0.26 <sup>Ba</sup>	11.18 ± 0.08 <sup>BCa</sup>
	UAE	11 ± 0.19 <sup>Aa</sup>	10.34 ± 0.02 <sup>Bb</sup>	12.53 ± 0.14 <sup>Cb</sup>	12.19 ± 0.10 <sup>Db</sup>	11.56 ± 0.10 <sup>Eb</sup>
MUFA	CP	16.02 ± 0.02 <sup>Aa</sup>	16.32 ± 0.08 <sup>Ba</sup>	16.59 ± 0.20 <sup>Ba</sup>	13.84 ± 0.09 <sup>Ca</sup>	14.36 ± 0.06 <sup>Da</sup>
	UAE	15.69 ± 0.04 <sup>Ab</sup>	16.85 ± 0.06 <sup>Bb</sup>	16.28 ± 0.08 <sup>Ca</sup>	13.58 ± 0.17 <sup>Da</sup>	13.49 ± 0.08 <sup>Db</sup>
PUFA	CP	73.33 ± 0.20 <sup>Aa</sup>	72.86 ± 0.04 <sup>Ba</sup>	71.85 ± 0.09 <sup>Ca</sup>	74.46 ± 0.11 <sup>Da</sup>	74.46 ± 0.13 <sup>Da</sup>
	UAE	73.4 ± 0.12 <sup>Aa</sup>	72.81 ± 0.04 <sup>Ba</sup>	71.19 ± 0.08 <sup>Cb</sup>	74.23 ± 0.15 <sup>Da</sup>	74.91 ± 0.10 <sup>Eb</sup>
UFA	CP	89.35 ± 0.02 <sup>Aa</sup>	89.18 ± 0.04 <sup>ABa</sup>	88.44 ± 0.13 <sup>CDa</sup>	88.3 ± 0.26 <sup>Da</sup>	88.82 ± 0.12 <sup>BCa</sup>
	UAE	89 ± 0.04 <sup>Ab</sup>	89.66 ± 0.12 <sup>Bb</sup>	87.47 ± 0.15 <sup>Cb</sup>	87.81 ± 0.10 <sup>Db</sup>	88.44 ± 0.16 <sup>Eb</sup>

Data are expressed as a mean value ± SD ( $n = 3$ ). Different letters indicate the significant difference ( $p < 0.05$ ) within column (lower case letters) and within rows (uppercase letters). SFA—saturated fatty acids, MUFA—monosaturated fatty acids, UFA—unsaturated fatty acids PUFA—Polyunsaturated fatty acids, CP—cold pressed oil; UAE—ultrasound assisted extraction, SE—Soxhlet extraction.

Total grape seed oil yield, obtained by Soxhlet method, ranged from 11.84% (Prokupac variety) to 15.77% (Cabernet sauvignon variety). Although the grape variety influenced the seed oil yield, more significant influence was observed for the applied type of the extraction technique. The yields of oil obtained by UAE were higher than those achieved by CP, with extraction efficiency, expressed as average value of oil recovering, of 74% and 85%, respectively. These results indicate that ultrasound positively affected the oil extraction, while CP gives lower oil yield than solvent extraction which is in agreement with already published results [33]. Yields obtained by UAE (10.06% to 13.01%) were comparable with the data reported by Da Porto et al. [34], but significantly higher than 9.5% found by Dimic et al. [5] for mixed samples of Cabernet Sauvignon, Merlot and Pinot Noir seeds with approximate ratio of 65:30:5 (m/m/m) or yield reported by Konuskan et al. [35] for Cabernet sauvignon (5.65%) and Merlot (5.65%) variety seeds. The differences between the grape seed oil yields may be ascribed to cultivation or extraction conditions, as well as to the climate, maturation stage and the grape seed separation moment during the fermentation.

Fatty acid composition of the obtained oils was similar in percentage regardless of grape variety or extraction method, but statistically significant differences can be found between contents of some fatty acids. Except for the Pinot Noir variety, saturated fatty acids (SFA) were found in significantly higher concentration ( $p < 0.05$ ) in UAE (10.34–12.53%), while higher concentration of unsaturated fatty acids (UFA) was found in oils obtained by CP (88.3–89.53%), except for Gamay variety. Slightly higher SFA content in oils obtained by UAE can be explained by the cavitation phenomenon and generation of locally extreme temperatures (5000 K) and pressures (1000 atm) in the cavitation zone. High energy

accumulation in the hot spots which is generated by the implosion of the cavitation bubbles, leads to high shear energy waves in the cavitation zone [36]. These conditions can provoke the oxidation of unsaturated fatty acids and breakdown of the polyunsaturated fatty acids since the double bonds can be oxidized by thermal effects [37,38]. Considering the higher content of UFA in oil obtained by CP, it mainly refers to the content of oleic acid and MUFA which is in accordance with the published results which stated that ultrasound and thermal processing caused the decrease of the oleic acid content in peanut, flaxseed and pomegranate seed oil [39,40]. It was stated that oil isolated from seeds of autochthonous variety Prokupac, by both methods, was characterized by the lowest content of polyunsaturated and the highest content of saturated fatty acids (Table 2). Regardless grape variety and method of extraction, the mayor fatty acid was linoleic and oleic acid. Determined values for linoleic acid were higher and for oleic lower than those reported by Konuskan et al. [35] for mechanically pressed method and varieties Merlot and Cabernet sauvignon (66.7%, 65.5%, respectively, for linoleic acid; 20.9%, 20.1% for oleic acid, respectively). In cold-pressed oils from Pinot Noir, Prokupac and Cabernet sauvignon varieties, the lower contents of total unsaturated fatty acids were observed compared to the UAE method which is in accordance with lower iodine values (Table 1). Lower iodine value indicates lower content of unsaturated fatty acids and the decrease of the iodine value can be a consequence of oxidation of double bonds caused by high temperature [41]. As already stated, the application of ultrasound can cause raise of the temperature and oxidation of double bonds. Lower content of UFA and lower iodine number was also observed for green coffee oil extracted by UAE suggesting that temperature affected the lower saturation of oil [37].

Linolenic fatty acid was found in small amounts in all tested oils, in the range from 0.28 to 0.31% in cold-pressed oils and from 0.26 to 0.36% in oils obtained by UAE. Compared to the results of other authors, those ranges were somewhat lower: Crews et al. [42] found linolenic acid in the range of 0.3–1.8%; Pardo et al. [3] in the range of 0.62–0.64%; and Rubio et al. [43] in the range of 0.35–0.97%. From the aspect of resistance to oxidation, the presence of linolenic acid is undesirable in large quantities. Since it is polyunsaturated, it is very susceptible to self-oxidation and could contribute to the unpleasant and undesirable smell and taste of oil [44]. A similar fatty acid profile in grape seed oils was reported by other authors [5,45]. Previously, similar fatty acid profile was published for Prokupac grape seed oils extracted by UAE from the fresh non-fermented dried pomace; however, significant differences among relative fatty acids contents were observed. This can be explained by the facts that the grape seed was collected from fresh, non-fermented material, but, also, by the differences in climate and grape growing conditions [46].

The results obtained in this study showed significant differences among the applied extraction methods and grape varieties in relation to the content of the most abundant fatty acids, while the lower effect of these factors on the content of minor fatty acids was observed.

### 3.2. Oxidative Status and Antioxidative Capacity of Oils

Autooxidation of vegetable oils is an unavoidable process that depends on the composition of the oil, storage conditions and the presence of components that accelerate or slow down this process [47]. Oxidation products (alcohols, aldehydes, ketones) which are formed, even in small quantities, give oils an unpleasant odor and taste and change their sensory properties [48]. For these reasons, determination of the oxidative status and oxidative stability of the oil was extremely important. Values of parameters related to oxidative status and oxidative capacity of oils are given in Table 3.

**Table 3.** Parameters of oxidative status and antioxidative capacity of oils.

Parameter	Extraction Method	Grape Variety				
		Pinot Noir	Gamay	Prokupac	Cabernet Sauvignon	Merlot
Peroxide value, mmol kg <sup>-1</sup>	CP	3.47 ± 0.058 <sup>Ca</sup>	3.07 ± 0.056 <sup>Ba</sup>	3.80 ± 0.00 <sup>Db</sup>	2.53 ± 0.062 <sup>Aa</sup>	3.10 ± 0.10 <sup>Ba</sup>
	UAE	4.10 ± 0.10 <sup>Bb</sup>	6.80 ± 0.00 <sup>Cb</sup>	4.47 ± 0.06 <sup>Aa</sup>	7.50 ± 0.00 <sup>Eb</sup>	7.07 ± 0.06 <sup>Db</sup>
Anisidine value, 100A <sup>1%</sup> <sub>350nm</sub>	CP	10.70 ± 0.00 <sup>Cb</sup>	10.33 ± 0.06 <sup>Aa</sup>	10.33 ± 0.06 <sup>Ab</sup>	11.07 ± 0.06 <sup>Db</sup>	10.50 ± 0.00 <sup>Ba</sup>
	UAE	10.13 ± 0.06 <sup>Aa</sup>	10.37 ± 0.06 <sup>Ca</sup>	10.20 ± 0.00 <sup>ABa</sup>	10.33 ± 0.06 <sup>BCa</sup>	11.03 ± 0.06 <sup>Db</sup>
Induction period, h	CP	2.82 ± 0.14 <sup>Ba</sup>	2.86 ± 0.02 <sup>Ba</sup>	2.79 ± 0.05 <sup>Ba</sup>	1.38 ± 0.01 <sup>Aa</sup>	1.30 ± 0.11 <sup>Aa</sup>
	UAE	8.09 ± 0.08 <sup>Db</sup>	7.11 ± 0.11 <sup>Cb</sup>	7.17 ± 0.08 <sup>Cb</sup>	5.48 ± 0.18 <sup>Bb</sup>	4.71 ± 0.04 <sup>Ab</sup>
OOT, °C	CP	166.80 ± 1.65 <sup>ABa</sup>	167.10 ± 1.61 <sup>Ba</sup>	166.90 ± 1.1 <sup>ABa</sup>	162.50 ± 2.23 <sup>ABa</sup>	162.20 ± 1.75 <sup>ABa</sup>
	UAE	184.30 ± 1.21 <sup>Db</sup>	179.67 ± 1.27 <sup>Cb</sup>	181.27 ± 1.55 <sup>DCb</sup>	173.80 ± 1.05 <sup>Bb</sup>	170.47 ± 1.03 <sup>Ab</sup>
EC <sub>50</sub> , mg mg <sup>-1</sup>	CP	52.97 ± 0.66 <sup>Bb</sup>	54.31 ± 0.76 <sup>Bb</sup>	43.32 ± 0.79 <sup>Ab</sup>	58.97 ± 1.12 <sup>Cb</sup>	65.18 ± 1.25 <sup>Db</sup>
	UAE	37.26 ± 1.25 <sup>Ba</sup>	38.66 ± 0.96 <sup>Ba</sup>	32.96 ± 0.77 <sup>Aa</sup>	42.25 ± 0.75 <sup>Ca</sup>	42.68 ± 0.56 <sup>Ca</sup>
α-tocopherol, mg 100 g <sup>-1</sup>	CP	1.47 ± 0.16 <sup>Ca</sup>	1.34 ± 0.04 <sup>Ba</sup>	1.67 ± 0.06 <sup>Ca</sup>	0.54 ± 0.26 <sup>Aa</sup>	1.06 ± 0.16 <sup>Ba</sup>
	UAE	2.32 ± 0.12 <sup>Bb</sup>	2.01 ± 0.15 <sup>Ab</sup>	2.77 ± 0.09 <sup>Cb</sup>	1.83 ± 0.09 <sup>Ab</sup>	2.43 ± 0.14 <sup>Bb</sup>
TPC, mg 100 g <sup>-1</sup>	CP	6.85 ± 0.23 <sup>Ba</sup>	7.18 ± 0.16 <sup>Ba</sup>	7.45 ± 0.09 <sup>Ca</sup>	6.28 ± 0.25 <sup>Aa</sup>	5.99 ± 0.34 <sup>Aa</sup>
	UAE	6.99 ± 0.27 <sup>BCa</sup>	7.24 ± 0.30 <sup>Ca</sup>	7.64 ± 0.28 <sup>Ca</sup>	6.35 ± 0.28 <sup>ABa</sup>	5.85 ± 0.27 <sup>Aa</sup>

Data are expressed as a mean value ± SD ( $n = 3$ ). Different letters indicate the significant difference ( $p < 0.05$ ) within column (lower case letters) and within rows (uppercase letters). OOT—initial oxidation temperature, TPC—total phenolic content, CP—cold pressed oil; UAE—ultrasound assisted extraction.

As it can be seen in Table 3, oxidative status and antioxidative capacity of oils depends on grape variety, but more intensely on extraction method applied. Peroxide and anisidine numbers indicated the presence of primary and secondary oxidation products. In terms of peroxide value, all oil samples were within the permissible limits prescribed by national quality regulation (7.5 mmol kg<sup>-1</sup>) [29], except for the oil from Cabernet sauvignon variety which was at the limit value. Statistical analysis of the values of peroxide and anisidine numbers revealed a significant difference both in terms of the applied extraction method and grape variety. The higher peroxide value of the grape seed oil samples obtained by UAE can be attributed to the oxidation reactions of fatty acids mainly by the action of free radicals which are formed under the ultrasound treatment [13]. This is in accordance with previously published results for peanut oil [49]. In addition, higher peroxide values in samples obtained by UAE can be expected bearing in mind the mechanism of UAE, which relies on the cavitation and mechanical effect and can cause the increase of the temperature which might induce oxidation of oils [49].

Oxidative stability or viability of vegetable oils represents the period during which they could be preserved from the intensive process of autooxidation. The results of determining the induction period based on the Rancimat test and determination of the initial oxidation temperature (OOT) by DSC are presented in Table 3. Determined values for the induction period indicated a much lower stability of cold-pressed oils, which was not affected by grape variety. Oils obtained with both methods from seed of Pinot Noir, Prokupac and Gamay varieties showed greater oxidative stability, while the ultrasound assisted method was found to be more efficient for recovery of oils with high oxidative stability (8.09 h, 7.11 h and 7.17 h, respectively). The literature data of the induction period of grape seed oil are quite different, primarily due to different test conditions (temperature and air flow). Madawala et al. [50] found that the induction period for grape seed oil was 8.9 h at a temperature of 100 °C and an air flow of 20 L h<sup>-1</sup>. The induction period in oil from seeds of different grape varieties at a temperature of 80 °C and an air flow of 7 L h<sup>-1</sup> was found in the interval from 19.7 to 40 h [51]. Results of oxidative stability determined for 27 different cold-pressed oil samples obtained at heating temperature of 100 °C and an air flow of 20 L h<sup>-1</sup>, showed large differentiation of induction time between samples, from 3.84 h to almost 40 h, for linseed oil and black cumin oil, respectively [52].



As lipid oxidation is an exothermic phenomenon, the resistance of the oil to oxidation was estimated on the basis of the determined starting point (onset point) of the exothermic DSC signal obtained during the oxidation of the oil in the controlled heating mode. OOT values were in the relatively narrow range for cold-pressed oils, from 162.20 to 167.10 °C and in a much wider range, from 173.8 to 184.3 °C for oils obtained by UAE.

The determined values of OOT also indicated lower oxidative stability of oils obtained by cold pressing, which was in accordance with the Rancimat test, as well as in terms of the varieties from which the more stable oils were extracted. A high correlation was found between these two methods for determining oxidative stability ( $R^2 = 0.99$ ).

Differences in fatty acids composition and content of total phenolic compounds affect differences in oxidative stability among different varieties. Pinot Noir, Prokupac and Gamay grape seed oils had a significantly higher content of monounsaturated oleic (C-18:1) and lower content of polyunsaturated linoleic acid (C-18:1) compared to Merlot and Cabernet sauvignon grape seed oils. The high degree of correlation between the TPC content and the values of the induction period ( $R^2 = 0.83$  for oils obtained by CP and  $R^2 = 0.74$  for oils obtained by UAE) confirms the great influence of TPC content on oxidative stability.

The scavenging activity of oil extracts was expressed as amount of sample needed to decrease the initial DPPH concentration by 50% ( $EC_{50}$ ). According to the results given in Table 3, cold pressed oils exhibited weaker efficiency to scavenge DPPH radicals compared to oils extracted using UAE extraction. An increase in scavenging activity among grape varieties was in following order: Prokupac > Pinot Noir > Gamay > Cabernet sauvignon > Merlot, for ultrasound isolated oils, while pressed oils were ranked as followed: Prokupac > Pinot Noir > Game > Cabernet Sauvignon > Merlot. Konuskan et al. [35] noted the highest DPPH scavenging activity for solvent extracted oils from variety Cabernet sauvignon. Due to the different methodology for determination of antioxidant activity, the results in our study could not be compared with other data in the literature [34,45,53].

Unrefined grape seed oils contain bioactive components including tocopherols and a number of phenolic compounds [20]. Grape seed oil is one of the main sources of vitamin E and it contains relatively high amounts of tocopherols and tocotrienols [54]. The high content of vitamin E and polyphenolic compounds contributes to the high stability of oil [7]. As can be seen in Table 3, the content of  $\alpha$ -tocopherol in cold-pressed oil samples was found in the range of 0.54 to 1.67 mg 100 g<sup>-1</sup>, while higher content was found in all oil samples obtained by UAE (range from 1.83 to 2.77 mg 100 g<sup>-1</sup>). Significantly higher  $\alpha$ -tocopherol content was published by Al Juhaimi et al. [55] for the grape seed oil obtained by CP (13.21 and 34.71 mg 100 g<sup>-1</sup>) and by Soxhlet extraction (8.89 and 33.89 mg 100 g<sup>-1</sup>). In the work of Dimic et al. [5], where UAE was applied as extraction method, the  $\alpha$ -tocopherol content was 6.51 mg 100 g<sup>-1</sup>, although the solvent-seed ratio was significantly higher compared to the one used in this paper (1:10 vs. 1:2). In samples of cold-pressed grape seed oil from the Brazilian market,  $\alpha$ -tocopherol content was present in the range 1.33 mg 100 g<sup>-1</sup>–1.76 mg 100 g<sup>-1</sup> [45]. Additionally, it should be noted that according to results in this study, both method of separation and grape variety had significant influence on the tocopherol content in the oils. As well as the good agreement with earlier published results of [56] found that application of ultrasound positively affects the content of tocopherols, chlorophylls and carotenoids in extracted olive oil.

Phenolic compounds have a great influence on the stability, sensory and nutritional characteristics of products and can spread their oxidative degradation by inactivation of free radicals [57]. In our work, no significant differences were observed for the TPC of grape seed oils extracted by both extraction methods, while among investigated varieties, the highest content of TPC was determined in oils obtained from Prokupac and Gamay seeds. By comparing the TPC values obtained in this study with the literature data it could be seen that they were higher than the results published by Bail et al. [20] ranging from 0.06 to 0.12 mg 100 g<sup>-1</sup>; and those published by Matthäus [58] and Pardo et al. [3], where determined range was from 1.07 to 3.44 mg 100 g<sup>-1</sup>. In cold-pressed oils from

Merlot and Cabernet sauvignon cultivars grown in eastern Mediterranean region of Turkey, a higher content of TPC was found compared to our study (15.1 and 18.2 mg 100 g<sup>-1</sup> respectively) [34]. The results obtained in this study confirmed the statement that the TPC value is under high influence on grape variety. The variations in TPC values of the grape seed oils originating from different grape varieties were in accordance with the content of TFC and  $\alpha$ -tocopherol content. Grape seed oils from grape varieties Merlot and Cabernet sauvignon had a lower content of these compounds compared to the oils from other grape varieties. It was reported that phenolic compounds showed high correlation with antioxidant activity [20,59]. Total phenolic content detected in cold pressed oils showed high and significant correlation with EC<sub>50</sub> values ( $R^2 = 0.76$ ), while correlation of  $R^2 = 0.53$  in oils extracted by UAE was calculated. A high degree of correlation was not found between the content of  $\alpha$ -tocopherol and the EC<sub>50</sub> values for both methods.

#### 4. Conclusions

The results presented in this work gives a different perspective of Prokupac grape usage by the means of grape pomace valorization. Despite the lower total oil content compared to the international grape seed, oil obtained from Prokupac grape seeds was better source of TPC and antioxidants which are known to have direct health benefits. Detailed characterization of oil from the Prokupac grape seeds showed that it belonged to oils that had enviable oxidative stability and antioxidant capacity. The results presented in this work also indicate that the methods chosen for the extraction of the oil could affect its quantity and quality. Ultrasound assisted method showed better yield and was more efficient to extract higher  $\alpha$ -tocopherol content and to present better antioxidant capacity by DPPH and oxidative stability by Rancimat test and DSC method.

The results reveal that UAE could be used for sustainable production of nutritionally valuable seed oil with high antioxidant activity from Prokupac grape pomace.

**Author Contributions:** Conceptualization, M.M. and J.M.; methodology, M.M.; software, M.M.; validation, V.R., B.D. and I.K.; formal analysis, J.M.; investigation, M.M.; resources, N.J.; data curation, J.M.; writing—original draft preparation, J.M.; writing—review and editing, I.K. and B.D.; visualization, B.D. supervision, I.K.; project administration, N.J.; funding acquisition, M.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Ministry of Agriculture, Forestry and Water Management, grant number 680-00-00067/1/2020-02 and Ministry of Education, Science and Technological Development of the Republic of Serbia grant number 451-03-9/2021-14/200133.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** The authors acknowledge Rubin A.D., Kruševac, Serbia for the materials used for experiments.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

#### References

1. Puglia, D.; Pezzolla, D.; Gigliotti, G.; Torre, L.; Bartucca, M.L.; Del Buono, D. The Opportunity of Valorizing Agricultural Waste, Through Its Conversion into Biostimulants, Biofertilizers, and Biopolymers. *Sustainability* **2021**, *13*, 2710. [[CrossRef](#)]
2. Spinei, M.; Oroian, M. The Potential of Grape Pomace Varieties as a Dietary Source of Pectic Substances. *Foods* **2021**, *10*, 867. [[CrossRef](#)]
3. Pardo, J.E.; Fernández, E.; Rubio, M.; Alvarruiz, A.; Alonso, G.L. Characterization of grape seed oil from different grape varieties (*Vitis vinifera*). *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 188–193. [[CrossRef](#)]

4. Martin, M.E.; Grao-Cruces, E.; Millan-Linares, M.C.; de la Paz, S.M. Grape (*Vitis vinifera* L.) Seed Oil: A Functional Food from the Winemaking Industry. *Foods* **2020**, *9*, 1360. [CrossRef] [PubMed]
5. Dimić, I.; Teslić, N.; Putnik, P.; Kovačević, B.D.; Zeković, Z.; Šojić, B.; Mrkonjić, Ž.; Colović, D.; Montesano, D.; Pavlič, B. Innovative and Conventional Valorizations of Grape Seeds from Winery By-Products as Sustainable Source of Lipophilic Antioxidants. *Antioxidants* **2020**, *9*, 568. [CrossRef]
6. Orsavova, J.; Misurcova, L.; Ambrozova, J.V.; Vicha, R.; Mlcek, J. Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. *Int. J. Mol. Sci.* **2015**, *16*, 12871–12890. [CrossRef]
7. 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]
8. Xia, L.; Xu, C.; Huang, K.; Lu, J.; Zhang, Y. Evaluation of phenolic compounds, antioxidant and antiproliferative activities of 31 grape cultivars with different genotypes. *J. Food Biochem.* **2019**, *43*, e12626. [CrossRef]
9. Kamal-Eldin, A. Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. *Eur. J. Lipid Sci. Technol.* **2006**, *58*, 1051–1061. [CrossRef]
10. Bjelica, M.; Vujasinović, V.; Rabrenović, B.; Dimić, S. Some Chemical Characteristics and Oxidative Stability of Cold Pressed Grape Seed Oils Obtained from Different Winery Waste. *Eur. J. Lipid Sci. Technol.* **2019**, *121*, 1–10. [CrossRef]
11. Ergović-Ravančić, M.; Obradović, V.; Mesić, J.S.; Marčetić, H.; Prtenjača, K.; Škrabal, S. The influence of grape seed drying temperature on the quality of grape seed oil. *J. Proc. Ener. Agri.* **2020**, *24*, 22–25. [CrossRef]
12. Nde, D.B.; Foncha, A.C. Optimization Methods for the Extraction of Vegetable Oils: A Review. *Processes* **2020**, *8*, 209. [CrossRef]
13. Chemat, F.; Rombaut, N.; Sicaire, A.G.; Meullemiestre, A.; Fabiano-Tixier, A.S.; Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason Sonochem.* **2017**, *34*, 540–560. [CrossRef] [PubMed]
14. Goula, A.M.; Ververi, M.; Adamopoulou, A.; Kaderides, K. Green ultrasound-assisted extraction of carotenoids from pomegranate wastes using vegetable oils. *Ultrason Sonochem.* **2017**, *34*, 821–830. [CrossRef]
15. Mushtaq, A.; Roobab, U.; Denoya, G.I.; Inam-Ur-Raheem, M.; Gullón, B.; Loorenco, J.M.; Francisco, B.J.; Zeng, X.A.; Wali, A.; Aadil, R.M. Advances in green processing of seed oils using ultrasound-assisted extraction: A review. *J. Food Process Preserv.* **2020**, *44*, e14740. [CrossRef]
16. Malićanin, M.; Rac, V.; Antić, V.; Antic, M.; Palade, L.M.; Kefalas, P.; Rakic, V. Content of Antioxidants, Antioxidant Capacity and Oxidative Stability of Grape Seed Oil Obtained by Ultra Sound Assisted Extraction. *J. Am. Oil Chem. Soc.* **2014**, *91*, 989–999. [CrossRef]
17. SRPS EN ISO 659:2011. *Seme Uljarica—Određivanje Sadržaja Ulja (Referentna Metoda)*; Institut za Standardizaciju Srbije: Belgrade, Serbia, 2011. (In Serbian)
18. IUPAC. Standard methods for the analysis of oils, fats and derivatives. In *International Union of Pure and Applied Chemistry*, 7th ed.; Paquot, C., Hautffenne, A., Eds.; Blackwell Scientific: Oxford, UK, 1992.
19. Ceci, L.N.; Carelli, A.A. Relation between oxidative stability and composition in Argentinian olive oils. *J. Am. Oil Chem. Soc.* **2010**, *87*, 1189–1197. [CrossRef]
20. Bail, S.; Stuebiger, G.; Krist, S.; Unterweger, H.; Buchbauer, G. Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity. *Food Chem.* **2008**, *108*, 1122–1132. [CrossRef] [PubMed]
21. Dabbou, S.; Brahmi, F.; Taamali, A.; Issaoui, M.; Ouni, Y.; Braham, M.; Zarrouk, M.; Hammami, M. Extra virgin olive oil components and oxidative stability from olives grown in Tunisia. *J. Am. Oil Chem. Soc.* **2010**, *87*, 1199–1209. [CrossRef]
22. Ulkowski, M.; Musialik, M.; Litwienko, G. Use of differential scanning calorimetry to study lipid oxidation. 1. Oxidative stability of lecithin and linolenic acid. *J. Agric. Food. Chem.* **2005**, *53*, 9073–9077. [CrossRef]
23. Vujasinovic, V.; Djilas, S.; Dimic, E.; Basic, Z.; Radocaj, O. The effect of roasting on the chemical composition and oxidative stability of pumpkin oil. *Eur. J. Lipid Sci. Technol.* **2012**, *114*, 568–574. [CrossRef]
24. Martinez, M.; Maestri, D. Oil chemical variation in walnut (*Juglans regia* L.) genotypes grown in Argentina. *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 1183–1189. [CrossRef]
25. AOCS Methods. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 6th ed.; AOCS Press: Champaign, IL, USA, 2017.
26. SRPS EN ISO 6883:2017. *Određivanje Konvencionalne Zapremenske Mase ("Litarske Mase na Vazduhu")*; Institut za Standardizaciju Srbije: Belgrade, Serbia, 2017. (In Serbian)
27. SRPS EN ISO 660:2011. *Masti i Ulja Životinjskog i Biljnog Porekla—Određivanje Kiselinskog Broja i Kiselosti*; Institut za Standardizaciju Srbije: Belgrade, Serbia, 2011. (In Serbian)
28. FAO/WHO. Codex Alimentarius Commission/FAO/WHO Food Standards. In *Standard for Named Vegetable Oils*, CODEX-STAN 210 ed.; FAO/WHO: Rome, Italy, 2011.
29. Pravilnik o Kvalitetu i Drugim Zahtevima za Jestiva Biljna Ulja i Masti, Margarin i Druge Masne Namaze, Majonez i Srodne Proizvode, "Sl. List SCG", br. 23/2006 i "Sl. Glasnik RS", br. 43/2013. Available online: <https://www.paragraf.rs/propisi/pravilnik-o-kvalitetu-ulja-masti-margarina-namaza-majoneza.html> (accessed on 13 September 2021). (In Serbian).
30. Morvarid, Y.; Leila, N.; Mohammad, G. Physicochemical properties of two type of shahrodi grape seed oil (Lal and Khalili). *Eur. J. Exp. Biol.* **2013**, *3*, 115–118.

31. Ozcan, M.M.; Endes, Z.; Er, F. Physical and Chemical Properties of Some Seed and Kernel Oils. *Asian J. Chem.* **2010**, *22*, 6531–6536.
32. Wang, T. Soybean oil. In *Vegetable Oils in Food Technology, Composition, Properties and Uses*; Gunston, F.D., Ed.; Blackwell Publishing Ltd.: Oxford, UK, 2002; pp. 43–44.
33. de Souza, R.C.; Machado, B.A.S.; Barreto, G.A.; Leal, I.L.; 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](#)]
34. Da Porto, C.; Porretto, E.; Decorti, E. Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds. *Ultrason. Sonochem.* **2013**, *20*, 1076–1080. [[CrossRef](#)]
35. Konuskan, D.B.; Kamiloglu, O.; Demirkeseer, 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.* **2019**, *53*, 144–149. [[CrossRef](#)]
36. Soria, A.C.; Villamiel, M. Effect of ultrasound on the technological properties and bioactivity of food: A review. *Trends Food Sci. Technol.* **2010**, *21*, 323–331. [[CrossRef](#)]
37. Dong, W.; Chen, Q.; Wei, C.; Hu, R.; Long, Y.; Zong, Y.; Chu, Z. Comparison of the effect of extraction methods on the quality of green coffee oil from Arabica coffee beans: Lipid yield, fatty acid composition, bioactive components, and antioxidant activity. *Ultrason Sonochem.* **2021**, *74*, 105578. [[CrossRef](#)]
38. Hernández-Santos, B.; Rodríguez-Miranda, J.; Herman-Lara, E.; Torruco-Uco, J.G.; Carmona-García, R.; Juárez-Barrientos, J.M.; Chávez-Zamudio, R.; Martínez-Sánchez, C.E. Effect of oil extraction assisted by ultrasound on the physicochemical properties and fatty acid profile of pumpkin seed oil (*Cucurbita pepo*). *Ultrason Sonochem.* **2016**, *31*, 429–436. [[CrossRef](#)]
39. Yu, H.; Seow, Y.X.; Ong, P.K.C.; Zhou, W. Effects of high-intensity ultrasound and oil type on the Maillard reaction of D-glucose and glycine in oil-in-water systems. *NPJ Sci. Food.* **2018**, *2*. [[CrossRef](#)]
40. Tian, Y.; Xu, Z.; Zheng, B.; Lo, Y.M. Optimization of ultrasonic-assisted extraction of pomegranate (*Punica granatum* L.) seed oil. *Ultrason Sonochem.* **2013**, *20*, 202–208. [[CrossRef](#)] [[PubMed](#)]
41. Alireza, S.; Tan, C.P.; Hamed, M.; Che Man, Y.B. Effect of frying process on fatty acid composition and iodine value of selected vegetable oils and their blends. *Int. Food Res. J.* **2010**, *17*, 295–302.
42. Crews, C.; Hough, P.; Godward, J.; Brereton, P.; Lees, M.; Guiet, S.; Winkelmann, W. Quantitation of the Main Constituents of Some Authentic Grape-Seed Oils of Different Origin. *J. Agric. Food Chem.* **2006**, *54*, 6261–6265. [[CrossRef](#)]
43. Rubio, M.; Alvarez-Orti, M.; Alvarruiz, A.; Fernandez, E.; Pardo, J.E. Characterization of Oil Obtained from Grape Seeds Collected during Berry Development. *J. Agric. Food Chem.* **2009**, *57*, 2812–2815. [[CrossRef](#)]
44. Baydar, G.N.; Akkurt, M. Oil content and oil quality properties of some grape seeds. *Turk. J. Agric. For.* **2001**, *25*, 163–168.
45. Shinagawa, F.B.; Santana, F.C.; Araujo, E.; Purgatto, E.; Mancini-Filho, J. Chemical composition of cold pressed Brazilian grape seed oil. *Food Sci. Technol.* **2018**, *38*, 164–171. [[CrossRef](#)]
46. Milinčić, D.D.; Kostić, A.Ž.; Špirović-Trifunović, B.D.; Tešić, Ž.L.; Tosti, T.B.; Dramićanin, A.M.; Barać, M.B.; Pešić, M.B. Grape seed flour of different grape pomaces: Fatty acid profile, soluble sugar profile and nutritional value. *J. Serbian Chem. Soc.* **2020**, *85*, 305–319. [[CrossRef](#)]
47. Martin-Polvillo, M.; Marquez-Ruiz, G.; Dobarganes, M.C. Oxidative stability of sunflower oils differing in unsaturation degree during long-term storage at room temperature. *J. Am. Oil Chem. Soc.* **2004**, *81*, 577–583. [[CrossRef](#)]
48. Gray, J.I. Measurement of lipid oxidation: A review. *J. Am. Oil Chem. Soc.* **1978**, *55*, 539–546. [[CrossRef](#)]
49. Zhang, L.; Zhou, C.; Wang, B.; Yagoub, A.A.E.; Ma, H.; Zhang, X.; Wu, M. Study of ultrasonic cavitation during extraction of the peanut oil at varying frequencies. *Ultrason Sonochem.* **2017**, *37*, 106–113. [[CrossRef](#)] [[PubMed](#)]
50. Madawala, S.R.P.; Kochhar, S.P.; Dutta, P.C. Lipid components and oxidative status of selected specialty oils. *Grasas Aceites.* **2012**, *63*, 143–151. [[CrossRef](#)]
51. Lutterodt, H.; Slavin, M.; Whent, M.; Turner, E.; Yu, L.L. Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. *Food Chem.* **2011**, *128*, 391–399. [[CrossRef](#)] [[PubMed](#)]
52. Symoniuk, E.; Ratusz, K.; Ostrowska-Ligeza, E.; Krygier, K. Impact of Selected Chemical Characteristics of Cold-Pressed Oils on their Oxidative Stability Determined Using the Rancimat and Pressure Differential Scanning Calorimetry Method. *Food Anal. Methods* **2018**, *11*, 1095–1104. [[CrossRef](#)]
53. Burčova, S.; Kreps, F.; Schmidt, Š.; Stržincova, P.; Jablonsky, M.; Kiselka, J.; Haz, A.; Šurina, I. Antioxidant activity and Tocopherol and Phenol contents of grape Residues. *BioResources* **2019**, *14*, 4146–4156. [[CrossRef](#)]
54. Freitas, L.S.; Jacques, R.A.; Richter, M.F.; Silva, A.L.; Caramao, E.B. Pressurized liquid extraction of vitamin E from Brazilian grape seed oil. *J. Chromatogr. A* **2008**, *1200*, 80–83. [[CrossRef](#)]
55. Al Juhaimi, F.Y.; Özcan, M.M. Effect of cold press and Soxhlet extraction systems on fatty acid, tocopherol contents, and phenolic compounds of various grape seed oils. *J. Food Process. Preserv.* **2017**, *42*, e13417. [[CrossRef](#)]
56. Jiménez, A.; Beltrán, G.; Uceda, M. High-power ultrasound in olive paste pretreatment. Effect on process yield and virgin olive oil characteristics. *Ultrason. Sonochem.* **2007**, *14*, 725–731. [[CrossRef](#)]
57. Siger, A.; Nogala-Kalucka, M.; Lampart-Szczapa, E. The content and antioxidant activity of phenolic compounds in cold-pressed plant oils. *J. Food Lipids* **2008**, *15*, 137–149. [[CrossRef](#)]
58. Matthäus, B. Virgin grape seed oil: Is it really a nutritional highlight? *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 645–650. [[CrossRef](#)]
59. Xu, C.; Yagiz, Y.; Hsu, W.Y.; Simonne, A.; Lu, J.; Marshall, M.R. Antioxidant, antibacterial and antibiofilm properties of polyphenols from muscadine grape (*Vitis rotundifolia* Michx.) pomace against selected foodborne pathogens. *J. Agric. Food Chem.* **2014**, *62*, 6640–6649. [[CrossRef](#)]