applied sciences

MDPI

Article

The Effectiveness of Extracts of Spent Grape Pomaces in Improving the Oxidative Stability of Grapeseed Oil

Miluska Cisneros [1], Elisa Canazza [1], Dasha Mihaylova [2] and Anna Lante [1,*]

[1] Department of Agronomy, Food, Natural Resources, Animals, and Environment—DAFNAE, Università di Padova, Viale dell'Università, 16, 35020 Legnaro, PD, Italy; miluskaalexandra.cisnerosyupanqui@phd.unipd.it (M.C.); elisa.canazza@unipd.it (E.C.)

[2] Department of Biotechnology, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria; dashamihaylova@yahoo.com

* Correspondence: anna.lante@unipd.it; Tel.: +39-049-8272920

Abstract: Spent grape pomace is a by-product of Grappa production that is usually considered waste. Therefore, in this study, in order to promote the sustainable use of by-products from the food industry, we aimed to optimize the extraction of antioxidants from spent grape pomace and their use to improve the oxidative stability of grapeseed oil. Ultrasound-assisted extraction maximized the total phenolic content and antioxidant activity, with the latter measured according to the ferric reducing antioxidant power. The best extraction conditions identified for spent grape pomace from red and white grapes were an amplitude of 40%, a duration of 22 min and a ratio of 1:37 and an amplitude of 40%, a duration of 25 min and a ratio of 1:45, respectively. Grapeseed oil, which is rich in polyunsaturated fatty acids and susceptible to rancidity, fortified with 10%, 20% and 30% of these extracts was evaluated in terms of its oxidative stability using the Rancimat method and compared with a control oil and an oil fortified with the synthetic antioxidant BHT at the highest legal level (200 ppm). For oil fortified with 30% of the extracts obtained from red and white pomace under the best conditions, increases in the induction time of 39% and 25% compared to the control and 23.01% and 10.62% compared to the BHT-fortified oil, respectively, were reported. This study highlights the potential of using grape pomace extracts as eco-friendly antioxidants to stabilize oil and contribute to the sustainability of the food industry at the same time.

Keywords: grape pomace; by-product valorization; antioxidants; oil stability; optimization

Citation: Cisneros, M.; Canazza, E.; Mihaylova, D.; Lante, A. The Effectiveness of Extracts of Spent Grape Pomaces in Improving the Oxidative Stability of Grapeseed Oil. *Appl. Sci.* **2024**, *14*, 10184. https://doi.org/10.3390/app142210184

Academic Editor: Ioannis G. Roussis

Received: 15 September 2024
Revised: 24 October 2024
Accepted: 28 October 2024
Published: 6 November 2024

1. Introduction

Spirit drinks are produced through alcoholic fermentation and distillation [1], and numerous kinds derived from post-vinification pomace, such as Orujo in Spain, Grappa in Italy, Tsipouro and Tsikoudia in Greece, Eaux de vie de marc in France, Aguardente Bagaceira in Portugal, Zivania in Cyprus and Törkölypálinka in Hungary [2], have been registered under geographical indications. According to EU Regulation 787/2019, the name "Grappa" is reserved exclusively for spirits obtained from grape pomace (GP) grown, vinified, distilled and processed in Italy [3,4]. Different types of Grappa are produced from different types of pomace [5,6]. Red pomace, which has already undergone fermentation, is ready for distillation, whereas pomace from white or rosé vinification, known as virgin or semi-fermented pomace, respectively, must be fully fermented before it is distilled [7]. In 2022, the Italian production of eaux de vie recorded significant growth, with a total of 95,410 hL of anhydrous alcohol produced, representing a 10% increase compared to 2021. Grappa production in particular spanned 83,000 hectares, marking a 12% increase in the area of production compared to that of the previous year [8]. Given the abundant production of Grappa in Italy, sustainable management of leftover residue is a key consideration. The spent grape pomace (SGP) generated in the Grappa distillation process is considered food waste and, thus far, has seldom been studied. In RP especially, the

total phenolic content (TPC) and antioxidant activity (AOA) are not altered significantly by this process [5,9]. Therefore, using SGP as a source of functional ingredients could be a promising approach to reduce the effect of its disposal on the environment and to valorize this by-product in innovative applications. Nowadays, trends in the use of mild technologies to extract bioactive compounds have emerged due to their many advantages over conventional methods. One of the main limitations to the valorization of by-products is the high cost of obtaining bioactive compounds. Consequently, innovative, economical and environmentally friendly extraction strategies must be used to exploit these precious resources. The extraction methods employed must ensure reducuctions in use of petroleum solvents, energy consumption and the generation of chemical waste to protect both the environment and consumers and guarantee the safety and high quality of the end products [10]. Green extraction methods include pressurized liquid extraction, supercritical fluid extraction, ultrasound-assisted extraction, deep eutectic solvent-assisted extraction, cold plasma-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction and electrical technologies [11–13]. These emerging techniques, when used in combination with bio-compatible solvents, such as water as a green solvent or a co-extractor, are considered viable and environmentally friendly alternatives for the recovery of natural compounds, preventing the formation of compounds that are toxic to humans and the environment [12]. Conversely, the traditional extraction methods used for many decades, such as Soxhlet extraction, maceration, infusion, decoction, percolation and hydrodistillation, require the use of toxic solvents and have higher energy requirements and longer processing times; harm the environment; have low selectivity and extraction yields; and may cause thermally sensitive components to decay [10,13]. In addition, the extracts obtained using these methods often require additional concentration and purification, thus extending the duration of analysis [12]. Among the emerging extraction techniques, ultrasound-assisted extraction (UAE) is accepted as an environmentally friendly option due to its high performance, lower solvent consumption and reduced duration, as well as its suitability for use with temperature-sensitive compounds [14]. UAE uses sound waves at a frequency (>20 kHz) able to generate collapsing cavitation bubbles, creating extreme local conditions. These phenomena cause fragmentation, erosion and sonoporation in plant tissues, which favors the release of bioactive compounds. Cavitation improves the absorption and diffusion of the solvent and, thus, increases the extraction yield. UAE also induces turbulence and shear force, causing cell walls to rupture and accelerating mass transfer. Together, these mechanisms contribute to the overall effectiveness of the process [11,14].

This work aimed to valorize white and red SGP, which are considered waste by-products of Grappa production. Antioxidant bioactive compounds were extracted from these materials using a water bath (WB), a water bath + ultrasound (WUS) and UAE, and the method that yielded the best results in terms of the total phenolic content (TPC) and ferric reducing antioxidant potential (FRAP) was optimized. Then, grapeseed oil was fortified with the extracts from grape pomace (EGPs), obtained under the optimum conditions, to delay its oxidation.

2. Materials and Methods

2.1. Materials and Chemicals

The Bonollo industrial distillery in Padua (Italy) provided the SGPs, constituting both white pomace (WP) and red pomace (RP), which are residual by-products of Grappa distillation. These pomaces were primarily derived from Chardonnay and Valpolicella (*Vitis vinifera*) grape varieties. The grapeseed oil used in this study was supplied by this same distillery.

Folin–Ciocalteu phenol reagent, gallic acid, ethanol, sodium carbonate, sodium hydroxide, hydrochloric acid, acetic acid, iron chloride, 2,3,5–triphenyltetrazolium (TPTZ), Butylated Hydroxytoluene (BHT) and Tetramethylchromane–2–carboxylic acid (Trolox) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other solvents and chemicals,

of analytical or MS grade, were purchased from Merck (Darmstadt, Germany) and Fisher Scientific (Fair Lawn, NJ, USA).

2.2. SGP Powder Preparation

Both SGPs were oven-dried at 50 °C for 48 h and then ground to a particle size < 500 µm using a water-cooled laboratory mill (IKA Werke M20, IKA-Werke GmbH & Co., Ltd., KG, Staufen, Germany). The powders obtained were stored in the dark at −18 °C until further analysis. The moisture content was evaluated using the oven-drying method at 105 °C for 24 h and recorded as percentages of 0.45% for the RP and 0.37% for the WP. All analytical determinations were carried out in triplicate and were expressed as the dry weight (dw).

2.3. The Extraction Procedure

In the preliminary phase of the study, the effects of using two different extraction techniques on the TPC of the extracts over time were compared: a conventional technique, using a WB, as reported in a previous study [15], and the emerging technique of UAE. Maceration was conducted at 50 °C for 30 min in the WB with constant stirring at 140 rpm. UAE was performed using a SONOPULS ultrasonic homogenizer (HD 2200.2, Bandelin, Berlin, Germany) at a frequency of 20 ± 0.5 kHz, with a KE 76 probe (Bandelin electronic GmbH & Co. KG, Berlin, Germany), for 30 min and at an amplitude of 25%. To avoid an increase in temperature as a result of the ultrasonic heating, the extraction system was placed in a beaker filled with ice, and the temperature was measured and monitored continuously in the range of 22–24 °C. In addition, ultrasonication cycles of 60 s with 10 s pauses in the middle were set in the apparatus to avoid the probe overheating. A third test was conducted combining the two techniques (WUS): the sample was subjected to maceration in the WB for the first 15 min, followed by UAE treatment for the remaining 15 min, with the same operating conditions maintained as those for the sole use of one of the techniques. In all the preliminary tests, samples of SGP dried and ground at a ratio of 1:10 w/v in 50% aqueous ethanol solution were used. At the end of the extraction, the solid residues were removed by centrifugation at 10,000× g for 5 min at 4 °C, and the supernatant was subsequently filtered using Whatman® N° 1 filter paper. Based on the results of the kinetic analysis of the TPC content and the AOA, as measured by FRAP, obtained by the three techniques, UAE was chosen as the method for subsequent optimization. The extracts obtained under the optimized conditions were vacuum-concentrated using a rotary evaporator (Rotavapor® R–100, Büchi, Essen, Germany) at a controlled temperature of 35 °C. The end volume of the slurry was 50% of the starting volume of the extract. The final extracts were subsequently used at different concentrations to fortify grapeseed oil containing 74.9% polyunsaturated fatty acids [16] and its oxidative stability evaluated using the official Rancimat method.

2.4. Experimental Design of Extract Optimization

For the experimental design of the RSM, amplitude (X1), ranging from 10% to 40%, time (X2), varying from 10 to 25 min, and the sample/solvent ratio (X3), set between 1:10 and 1:50, were selected as variables. A Box–Behnken design (BBD) was used to evaluate the influence of these variables on the TPC and FRAP of both types of SGP. The results obtained were correlated with the experimental factors using a second-order polynomial function (Equation (1)).

$$Y_i = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_{ii}^2 + \sum_{i<j} \beta_{ij} X_i X_j \quad (1)$$

where Y_i is the dependent variable, β_0 is the intercept, β_i are the linear coefficients, β_{ii} represent the quadratic terms for the curvature effects and β_{ij} are the interaction coefficients

accounting for the combined effects of different experimental factors X_i and X_j, which represent the levels of the independent variables.

In addition to the use of the RSM, the data were presented as the mean ± standard deviation of three separate and independent experiments (n = 3).

2.5. Analytical Determinations

2.5.1. Total Phenolic Content (TPC)

The TPC was measured using the Folin–Ciocalteu method, as reported in the literature [17]. In brief, 500 µL of the diluted sample was combined with 250 µL of 1 N Folin–Ciocalteu reagent and 1250 µL of 7.5% $NaCO_3$ solution. As a control, a white solution with water was used instead of the sample. After storing it in the dark for 30 min, the absorbance of the sample was measured at 755 nm using a Varian Carry 50 Bio UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). These results were expressed in mg of Gallic Acid Equivalent (GAE) per gram of dw (mg GAE/g dw).

2.5.2. Ferric Reducing Antioxidant Potential (FRAP)

Antioxidant activity (AOA) was determined using the ferric reducing antioxidant potential (FRAP) method, as described in the literature [18]. The FRAP solution was prepared by mixing 2.5 mL of 0.01 M TPTZ into 40 mM HCl, 2.5 mL of 0.02 M $FeCl_3$ aqueous solution and 25 mL of 0.2 M sodium acetate buffer. Subsequently, 100 µL of the sample was added to 900 µL of the FRAP solution, and the mixture was incubated at 37 °C for 30 min. A white solution was also prepared using the dilution solvent. Absorbance was measured at 593 nm using a Varian Carry 50 Bio UV/Vis spectrophotometer. These results were expressed in mg of Trolox Equivalent (TE) per g dw (mg TE/g dw).

2.5.3. Oxidative Stability

Grapeseed oil was fortified with the EGPs derived from the RP and WP under the optimal ultrasound-assisted extraction (UAE) conditions. Concentrations of 10%, 20% and 30% of the EGPs from both the RP and the WP were used to fortify the oil, and its oxidative stability was compared with that of the control (grapeseed oil only). As a benchmark, BHT was used as a synthetic antioxidant at a concentration of 200 ppm, aligning with the maximum level permitted by the Codex Alimentarius 2023 [19]. The optimized EGPs were mixed with grapeseed oil using ultrasonic treatment at an amplitude of 25% for 2 min and 30 s split into 30 s cycles. Subsequently, the samples were homogenized with an Ultra Turrax T–25 (Janke and Kunkel, Staufen, Germany) at a speed of 12,000 rpm for 5 min. The samples (3 g each) were then analyzed for their oxidative stability using the official Rancimat method at 110 °C and using an air flow of 20 L/h. Oxidative stability was measured according to the induction time (IT), which indicates the time passed (expressed in hours) before the conductivity of water (µS/min) begins to increase due to the formation of secondary compounds derived from lipid oxidation. The Antioxidant Activity Index (AAI) was calculated using the following formula (Equation (2)):

$$AAI = \frac{IT\ of\ oil\ with\ antioxidants}{IT\ of\ oil\ without\ antioxidants(control)} \quad (2)$$

2.5.4. Statistical Analysis

Experimental and statistical analyses were performed using Statgraphics® Centurion XIX software, version 19.3.03 (StatPoint Technologies, Inc., Warrenton, VA, USA). A BBD was used to evaluate the influence of the variables on the optimization process across fifteen experiments. The patterns and regressions were considered significant at the level of $p < 0.05$. All data were reported as the mean ± standard deviation of three separate and independent experiments (n = 3).

3. Results and Discussion

3.1. Extraction of Antioxidant Bioactive Compounds

As reported in Figures 1 and 2, UAE caused a rapid increase in the TPC values in both types of SGP, obtaining values of 35.55 ± 0.36 mg GAE/g dw for the WP and 27.35 ± 0.57 mg GAE/g dw for the RP after the first 15 min of treatment. In monitoring the TPC content extracted from GP using UAE at different amplitudes, Da Rocha and Noreña (2019) highlighted that ultrasonic waves act in the first few minutes of extraction, where the cavitational effects in the solvent near the plant materials form microchannels towards the food matrix, resulting in greater penetration. However, over time, the ultrasonic waves exert increased action in breaking down the cell structures, causing some of the extracted constituents to be lost, which can affect the mass transfer capacity, thus decreasing the permeability of the cellular structures to the solvent [20]. The combined WUS treatment led to a major increase in the TPC only in the second half of the treatment duration, i.e., when UAE was introduced. The WUS treatment achieved a TPC that was only slightly higher than that yielded by using UAE for 25 min and 27.5 min for the WP and the RP, respectively, but these differences were not significant ($p < 0.05$). A comparison of the extraction methods based on statistical analysis is reported in Table S1. When the temperature increases, the yield increases due to the higher porosity of the material; higher solvation; a reduction in the surface tension and viscosity of the extracts; and greater mass transfer. However, a temperature higher than the classic range of 20 to 50 °C conventionally used in the extraction of polyphenols by maceration can easily lead to hydrolysis and oxidation of many phenolic compounds, especially over prolonged extraction times [21].

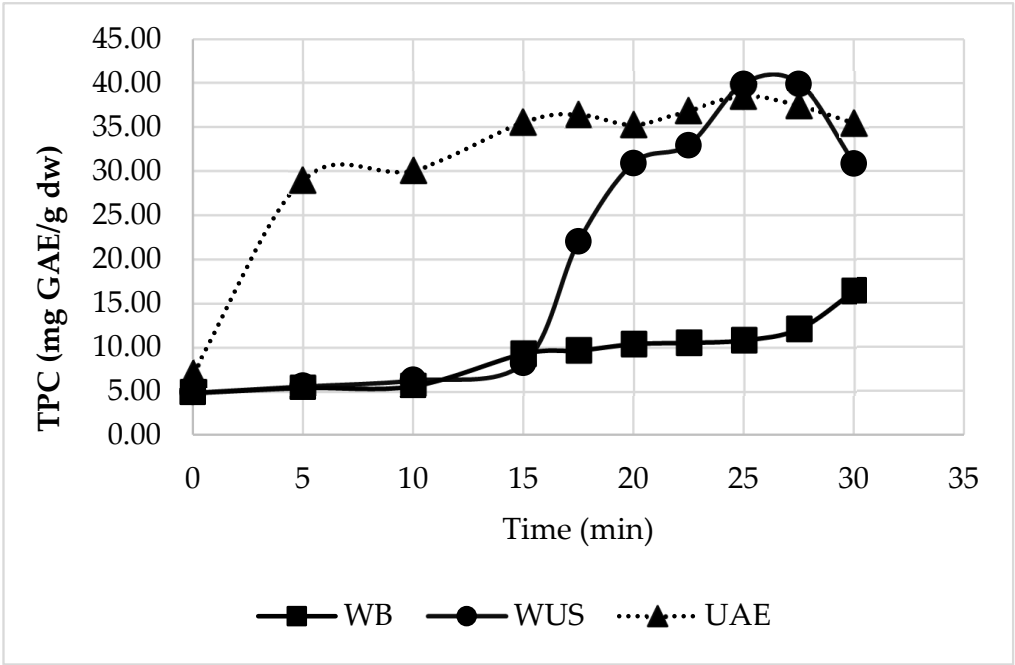


Figure 1. Kinetics over time of TPC during hydroalcoholic extraction of bioactive compounds from WP using WB, WUS and UAE methods.

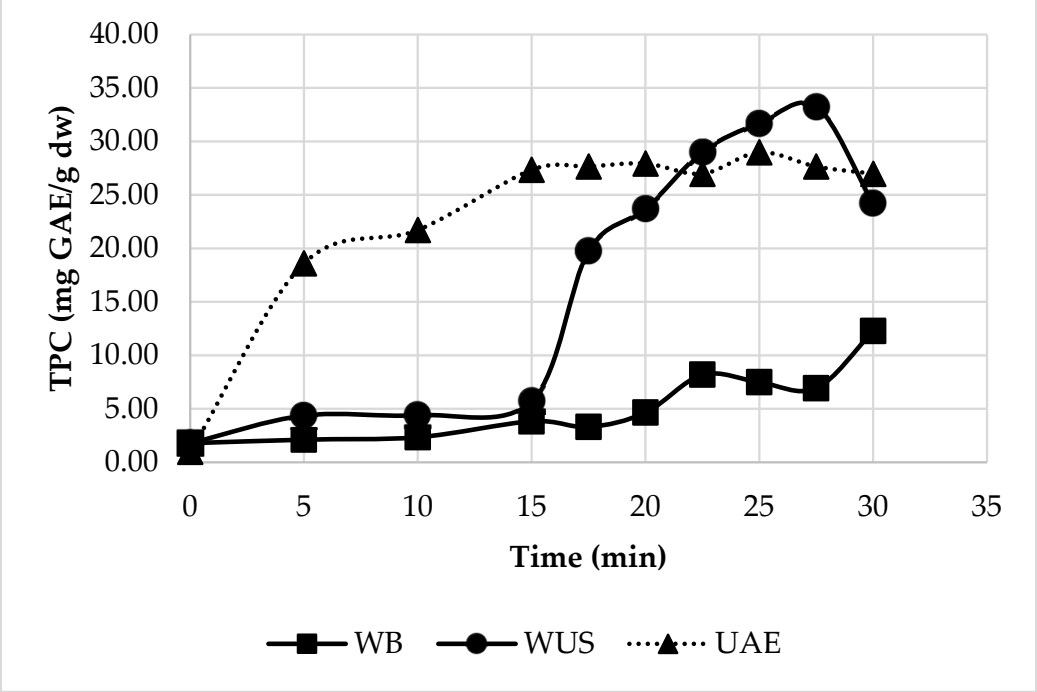


Figure 2. Kinetics over time of TPC during hydroalcoholic extraction of bioactive compounds from red grape pomace (RP) using WB, WUS and UAE methods.

Several emerging technologies, including UAE, are more suitable for the extraction of thermolabile substances because they do not primarily exploit thermal energy for extraction [22–25]. An increase in temperature reduces the intensity of cavitation due to lower surface tension and increased vapor pressure in the cavitation bubbles and, therefore, may reduce the extraction rate. Conducting processes at ambient temperature and pressure also reduces the energy requirements and costs of processing [26]. UAE does not use hazardous chemicals, offers a shorter extraction time and has a high energy efficiency and extraction rate [27]. González-Centeno et al. [28] showed that UAE (55 ± 5 kHz, 435 ± 5 W/L, cycles of 0.5 s) could extract the total phenolic compounds from RP eight times faster than conventional mechanical agitation (50 °C, 200 rpm). A study by Thais et al. (2017) [29] corroborated this finding, showing that UAE (20 kHz, 1000 W/L, 28 ± 3 °C) enabled a significantly higher recovery of the TPC in a shorter time compared to both microwave-assisted extraction and conventional mechanical stirring. The TPC in the extracts obtained using UAE was twice as high as that in those obtained using conventional mechanical stirring just 9 min after the start of extraction, demonstrating its superior effectiveness for the recovery of total phenolic compounds.

In the present study, in the first 10 min, the TPC extracted from the WP was more than five times higher when using UAE than when applying conventional extraction with the WB, while values more than nine times higher were obtained for the RP. For both SGPs, at 30 min, the TPC obtained using UAE was just over double that when using the WB.

The improvement of the processing efficiency in using sonication is mainly attributed to acoustic cavitation and its consequent localized thermal and mechanical effects, namely increased mass transfer and significant cell wall breakdown, offering higher extraction yields and significantly reduced processing times compared to conventional techniques [30]. The impact of UAE on the cell structure accelerates both swirling and internal diffusion. This enhanced diffusion allows the solvent to effectively penetrate the cells and extract the desired compounds from the inside [14]. The most common solvents used to extract phenolic compounds are methanol, ethanol, acetone and water [31,32]. However, it has been shown that aqueous solvent mixtures are much more effective than pure solvents [33]. Ethanol, chosen as the co-extractant in this study, is a widely used solvent for the extraction

of phenolic compounds from GP. It is suitable; non-toxic to researchers and the environment; economical; and already used in the winemaking process, underscoring its importance in the extraction process [12,34–37]. On these bases, we opted to optimize UAE of the bioactive compounds.

3.2. Optimization of UAE of the Antioxidant Bioactive Compounds

A BBD was used to optimize the UAE, considering three factors: amplitude, time and the sample/solvent ratio. Fifteen runs of the experiments were conducted (Table 1).

Table 1. The TPC (mg GAE/g dw) and FRAP (mg TE/g dw) of the RP and the WP during the optimization of ultrasound-assisted extraction according to a Box–Behnken design of experiments under different experimental conditions: amplitude, % (X1); time, min (X2); sample/solvent ratio (X3).

				RP		WP	
Runs	**X_1**	**X_2**	**X_3**	**TPC**	**FRAP**	**TPC**	**FRAP**
1	25	17.5	1:30	26.60 ± 1.78	78.98 ± 4.85	31.07 ± 2.94	86.76 ± 1.61
2	10	17.5	1:10	25.84 ± 0.66	53.31 ± 1.84	29.25 ± 0.84	36.17 ± 3.08
3	25	10	1:10	27.50 ± 1.22	54.77 ± 2.37	32.95 ± 2.03	58.00 ± 13.70
4	25	25	1:10	3.69 ± 0.58	14.45 ± 0.75	32.82 ± 4.50	50.61 ± 2.98
5	25	10	1:50	18.83 ± 0.11	77.90 ± 4.90	21.91 ± 2.36	48.78 ± 7.47
6	25	25	1:50	24.80 ± 5.02	89.75 ± 1.82	34.83 ± 0.91	88.23 ± 3.23
7	10	17.5	1:50	8.74 ± 1.33	63.39 ± 0.51	16.05 ± 0.92	55.32 ± 2.61
8	25	17.5	1:30	33.61 ± 0.93	77.86 ± 4.96	35.44 ± 5.89	89.17 ± 2.50
9	40	10	1:30	32.99 ± 1.36	82.03 ± 1.01	40.63 ± 0.39	101.24 ± 0.64
10	40	17.5	1:50	28.95 ± 0.56	81.99 ± 7.70	35.93 ± 4.98	98.32 ± 1.57
11	40	17.5	1:10	33.80 ± 0.83	64.39 ± 4.61	38.24 ± 1.09	86.57 ± 7.06
12	10	25	1:30	20.73 ± 3.73	57.52 ± 9.44	17.63 ± 0.15	64.16 ± 1.20
13	40	25	1:30	48.48 ± 5.24	105.64 ± 8.41	44.68 ± 2.96	102.59 ± 1.99
14	10	10	1:30	11.42 ± 1.56	50.62 ± 4.34	27.88 ± 1.64	87.09 ± 0.93
15	25	17.5	1:30	34.79 ± 2.60	94.59 ± 3.56	29.05 ± 1.70	79.51 ± 4.83

The TPC obtained from the RP and the WP in these experiments ranged from 3.69 to 48.48 mg GAE/g dw and 16.05 to 44.68 mg GAE/g dw, respectively, while the FRAP ranged from 14.45 to 105.64 mg TE/g dw and 36.17 to 102.59 mg TE/g dw, respectively. The highest TPC and FRAP were obtained at an amplitude of 40%, using an extraction time of 25 min and at a sample/solvent ratio of 1:30 for both types of SGP.

For all variable responses obtained in the experimental matrix, Table 2 shows the results of conducting a regression analysis and an ANOVA in adapting the model design to determine whether the terms were statistically significant.

As shown in Table 3, for the RP, amplitude, quadratic interactions (AA) and ratio–time interactions (AC) significantly influenced ($p < 0.05$) both the TPC and the FRAP. The sample/solvent ratio also significantly affected the FRAP ($p < 0.05$). For the WP, the amplitude had a significant effect ($p < 0.05$) on both the TPC and the FRAP. In terms of FRAP, for WP as well as RP, the sample/solvent ratio, quadratic interactions (AA) and ratio–time interactions (AC) also had significant effects ($p < 0.05$). Indeed, other recent studies on the optimization of the extraction parameters in UAE for the recovery of polyphenolic compounds from plant matrices and by-products have also reported significant ($p < 0.05$) impacts of factors such as the amplitude used and/or time–ratio interactions [38–41]. The second-order predictive polynomial equations obtained by applying quadratic regression models to the experimental values to represent the empirical relationship between the TPC and the FRAP and the operating conditions are shown below (Equations (3)–(6)):

RP

$$TPC = 14.8945 + (0.0945833 \cdot Ratio) - (0.163824 \cdot Amplitude) + (1.05176 \cdot Time - 0.0212917 \cdot (Ratio)^2 + (0.0102042 \cdot Ratio \cdot Amplitude) + (0.049625 \cdot Ratio \cdot Time) + 0.00526481 \cdot \left(Amplitude \right)^2 + (0.0137333 \cdot Amplitude \cdot Time) - 0.0790741 \cdot \left(Time \right)^2 \quad (3)$$

$$FRAP = 17.2539 + (1.56819 \cdot Ratio) + (0.439769 \cdot Amplitude) + (1.60485 \cdot Time) - 0.0409677 \cdot (Ratio)^2 + (0.00626667 \cdot Ratio \cdot Amplitude) + (0.0869333 \cdot Ratio \cdot Time) - 0.00734259 \cdot (Amplitude)^2 + (0.0371111 \cdot Amplitude \cdot Time) - 0.145904 \cdot (Time)^2 \quad (4)$$

WP

$$TPC = 52.3174 - (0.4568 \cdot Ratio) - (0.266311 \cdot Amplitude) - (1.83954 \cdot Time) - 0.00506735 \cdot (Ratio)^2 + (0.00907594 \cdot Ratio \cdot Amplitude) + (0.0217452 \cdot Ratio \cdot Time) + 0.000199704 \cdot (Amplitude)^2 + (0.0317853 \cdot Amplitude \cdot Time) + 0.0143494 \cdot \left(Time \right)^2 \quad (5)$$

$$FRAP = 56.3087 + (2.41467 \cdot Ratio) - (0.799285 \cdot Amplitude) - (2.24923 \cdot Time) - 0.0542652 \cdot (Ratio)^2 - (0.0061721 \cdot Ratio \cdot Amplitude) + (0.0780597 \cdot Ratio \cdot Time) + 0.0251357 \cdot (Amplitude)^2 + (0.0539508 \cdot Amplitude \cdot Time - 0.0361915) \cdot (Time)^2 \quad (6)$$

Table 2. Regression coefficients and F-ratios of the predicted second-order polynomial models for TPC and FRAP obtained from the RP and the WP.

	RP				WP			
	TPC		FRAP		TPC		FRAP	
	Regression Coefficients	F-Ratio	Regression Coefficients	F-Ratio	Regression Coefficients	F-Ratio	Regression Coefficients	F-Ratio
Constant	14.8945	–	17.2539	–	52.3174	–	56.3087	–
A: Ratio	0.0945833	1.06	1.56819	39.97 *	−0.4568	7.38	2.41467	7.96 *
B: Amplitude	−0.163824	70.45 *	0.439769	29.98 *	−0.266311	57.75 *	−0.799285	48.28 *
C: Time	1.05176	0.57	1.60485	0.01	−1.83954	0.53	−2.24923	0.25
AA	−0.0212917	25.13 *	−0.0409677	19.94 *	−0.00506735	1.49	−0.0542652	31.53 *
AB	0.0102042	3.52	0.00626667	0.28	0.00907594	2.91	−0.0061721	0.25
AC	0.049625	20.80 *	0.0869333	13.68 *	0.0217452	4.17	0.0780597	9.94 *
BB	0.00526481	0.49	−0.00734259	0.20	0.00019970	0.00	0.0251357	2.14
BC	0.0137333	0.90	0.0371111	1.40	0.0317853	5.01	0.0539508	2.67
CC	−0.0790741	6.85	−0.145904	5.00	0.0143494	0.24	−0.0361915	0.28

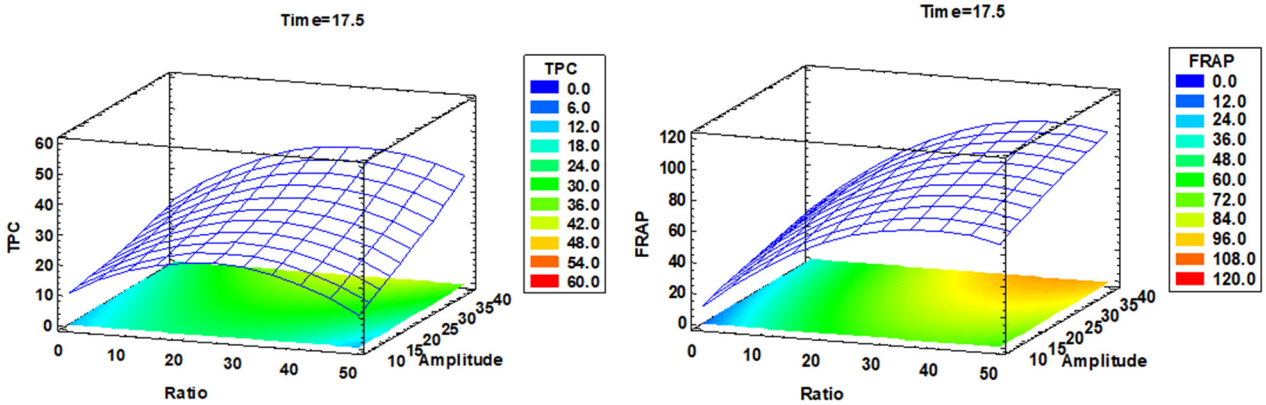
* $p < 0.05$.

The optimal theoretical values in terms of TPC and FRAP were obtained using a combination of a ratio of 1:37, an amplitude of 40% and a time of 22 min on the RP (43.57 mg GAE/g dw and 102.57 mg TE/g dw, respectively) and a ratio of 1:45, an amplitude of 40% and a time of 25 min on the WP (46.74 mg GAE/g dw and 115.58 mg TE/g dw, respectively). To verify the validity of the model, these conditions were experimentally tested, obtaining a TPC and FRAP of 32.70 ± 0.68 mg GAE/g dw and 84.16 ± 1.53 mg TE/g dw for the RP and 37.23 ± 4.29 mg GAE/g dw and 95.27 ± 2.87 mg TE/g dw for the WP, respectively.

The R^2 values observed for the TPC (0.73 for the RP and 0.84 for the WP) and the FRAP (0.77 for the RP and 0.84 for the WP) indicate that the model has a discrete predictive ability. However, there is room for improvement to optimize the model's accuracy even further. Ideal R^2 values should be at least above 0.75, and values above 0.90 represent a well-fitted model [41–43]; however, numerous previous studies have shown that models with an R^2 value of less than 0.90 can still explain the experimental variability in the response variables satisfactorily [44–47]. Although the R^2 and Adj-R^2 values suggest that the accuracy of the model can be improved, the results on the F-ratio and the lack of fit are not significant

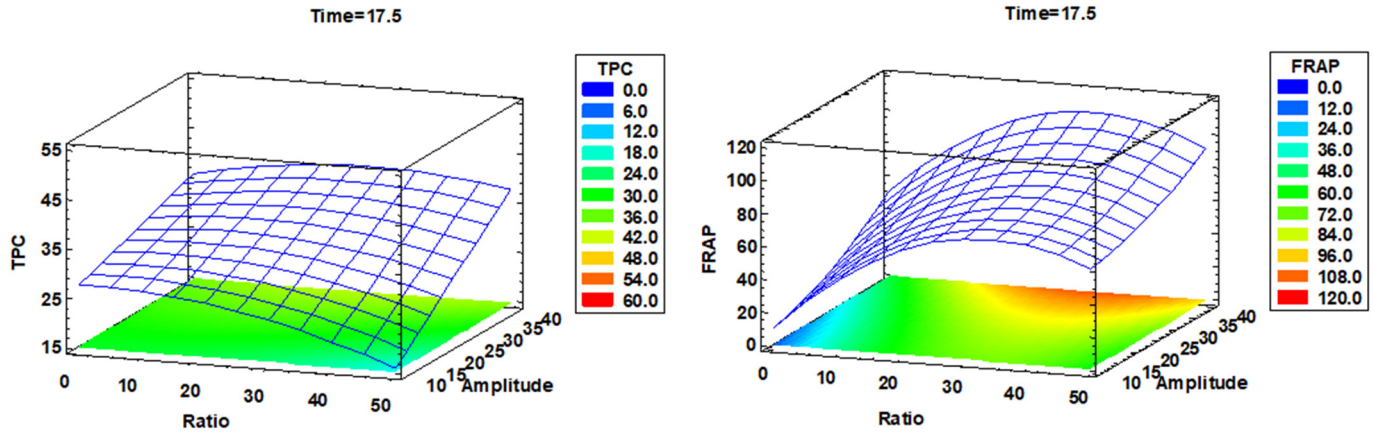
($p > 0.05$), indicating that the model possesses a reliable predictive capacity and fits the experimental data.

Figures 3 and 4 show that when amplitude increases up to 40%, the bioactivity steadily increases. At high amplitudes, an important release of phenolic compounds occurs; however, the extraction yield reduces after a given peak, beyond which points polyphenols are destroyed [41,48,49]. Another previous study also showed that polyphenols can be damaged at amplitudes greater than 40% [50]. However, the optimal amplitude depends on the matrix and the stability of its antioxidant molecules. The amplitude at which the highest FRAP was obtained was 80% for fortified *Bauhinha* [51], 90% for sugar beet molasses [52], 50% for pomegranate [53] and 30% for areca nuts [54]. Another important determinant of the FRAP is the solute/solvent ratio, which was optimal at 1:37 and 1:45 for the RP and the WP, respectively. Increases in the TPC and FRAP with a decrease in the solid/solvent ratio correspond to an increase in the yield of polyphenols, probably due to better swelling of the solute powder, which favors the cavitation process and causes cell wall rupture and more efficient mass transfer during UAE [55–58]. Previous research has determined that the extraction yields of polyphenolic compounds for different materials depend on the properties and structure of the compounds, the degree of polymerization and its relationship with the polarity of the solvent used for extraction [59].



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

Figure 3. The response surface for TPC (**a**) and FRAP (**b**) when using UAE on RP. In all cases, the third variable (C = time) is taken as the mean value.



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

Figure 4. The response surface for TPC (**a**) and FRAP (**b**) when using UAE on WP. In all cases, the third variable (C = time) is taken as the mean value.

Table 3. Analysis of variance for the response surface quadratic models of TPC and FRAP.

	RP								WP							
	TPC				FRAP				TPC				FRAP			
	Degrees of Freedom	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F-Ratio**	**Degrees of Freedom**	**Sum of Squares**	**Mean Square**	**F-Ratio**
Lack of fit	15	908.701	60.5801	2.84 *	15	2921.04	194.736	1.96 *	15	207.78	13.852	0.68 *	15	1834.02	122.268	1.11 *
Pure error	4	85.2637	21.359		4	397.862	99.4655		4	81.6453	20.411		4	441.37	110.342	
Total (corr.)	29	3743.76			29	14,279.3			29	1913.65			29	13,872.3		
R^2		0.73				0.77				0.84				0.84		
Adj-R^2		0.59				0.65				0.75				0.75		

* $p > 0.05$.

The analysis of variance showed that the extraction time did not significantly influence ($p > 0.05$) the TPC or FRAP. However, the ratio–time interaction had a significant effect ($p < 0.05$) on both variables for the RP and on FRAP only for the WP. The TPC and FRAP improved when the extraction time and solvent ratio were increased [60].

3.3. Determination of the Oxidative Stability

Table 4 shows the oxidative stability of the grapeseed oil fortified with the WP and RP extracts with the optimal TPC and FRAP values at different concentrations (10%, 20% and 30% *w*/*w*). Unaltered rapeseed oil samples and samples fortified with a conventional synthetic antioxidant (BHT at 200 ppm) were evaluated as the controls.

Table 4. Oxidative stability of grapeseed oil fortified with the optimum WP and RP extracts measured using the Rancimat test.

Sample	WP		RP	
	IT (h)	AAI	IT (h)	AAI
Grapeseed oil	4.65 ± 0.16^{Bc}	1.00	4.65 ± 0.16^{Ac}	1.00
Grapeseed oil + BHT at 200 ppm	5.24 ± 0.14^{Bb}	1.13	5.24 ± 0.14^{Ab}	1.13
Grapeseed oil + 10% SGP extract	4.82 ± 0.14^{Bbc}	1.03	5.26 ± 0.53^{Abc}	1.13
Grapeseed oil + 20% SGP extract	5.32 ± 0.02^{Bb}	1.14	5.69 ± 0.57^{Ab}	1.22
Grapeseed oil + 30% SGP extract	5.79 ± 0.23^{Ba}	1.25	6.46 ± 0.88^{Aa}	1.39

Data are expressed as the mean ± standard deviation ($n = 3$). Different letters indicate statistically significant differences ($p < 0.05$) between the type of SGP (capital letters) and the type of sample fortified (lowercase letters), according to ANOVA (two-way) and Duncan's test.

Previous studies have indicated that it is possible to increase the induction time (IT) of edible oils by adding antioxidant extracts derived from various plant by-products to them and obtain high value-added products with an improved oxidative stability and shelf life [61–66].

As shown in Table 4, the grapeseed oil without any fortification (the control) had the lowest oxidation stability, with an IT of 4.65 ± 0.16 h. The grapeseed oils fortified with BHT and with red SGP at 10% increased in their stability by 13% ($p < 0.05$). In addition, using the red SGP provided a higher oxidative stability than using the white SGP at all the concentrations tested. However, using either RP or WP, the AAI achieved was much higher ($p < 0.05$) than that of the control, and when using BHT, in particular when fortifying the oil with 30% of the extracts, increases over the control of 25% and 39% were obtained for WP and RP, respectively.

Sonication treatment was applied to mix the different extracts with the grapeseed oil, improving the transfer of antioxidant components from the extract to the oil and obtaining a more homogeneous distribution of antioxidant compounds [41,67]. As reported by Zacometti et al. (2024), the use of sonication to increase the solubility and miscibility of extracts in oils can increase the presence of structured phenolic lipids, which work as natural antioxidants [68]. These data suggest that the optimal sample should have a prolonged IT, with this effect being more evident when increasing the concentration of the extract. However, the extracted antioxidant compounds, as well as delaying the oxidation of the oil, may exert additional bioactivity [69]. Furthermore, interactions between these antioxidant compounds and other molecules present in the matrix may have a positive or negative impact on the oxidative stability of food systems, resulting in synergism or antagonism [70]. Future studies should provide a detailed metabolomic profile of the extracts obtained for a fuller understanding of their bioactivity. In addition, monitoring the fatty acid profile of fortified grapeseed oil during storage is crucial to assessing how these extracts affect its stability over time. The industrial scalability and cost-effectiveness of using these natural extracts and their potential interactions with other components of food will need to be assessed. Finally, sensory evaluations and studies on consumer acceptance are required.

4. Conclusions

This study explored the recovery of a by-product of the Grappa industry, SGP, using UAE. This approach falls under the context of a circular economy, in which by-products from the wine industry are transformed into precious resources, contributing to environmental sustainability. UAE was shown to improve the extraction of antioxidant bioactive compounds, achieving superior results in terms of the TPC more quickly than when using conventional methods, such as a WB and the combined WUS method. Grapeseed oil fortified with 30% EGPs experienced significantly delayed oxidation compared to that seen when adding the synthetic antioxidant BHT at the highest level allowed by law (200 ppm). Therefore, using these extracts as natural antioxidants represents a valid alternative to the use of synthetic antioxidants such as BHT, reducing the environmental impact and promoting greater sustainability in the food industry. Future studies should focus on an in-depth metabolomics characterization of the optimized extracts to clarify their composition and identify the bioactive compounds responsible for their intriguing antioxidant activity. In addition, the fatty acid profile of the fortified oil during storage must be monitored in future to ensure its long-term stability.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app142210184/s1, Table S1. Total phenolic content (mg GAE/g) of grape pomace after distillation along the extraction time.

Author Contributions: Conceptualization, M.C. and A.L.; methodology, M.C. and E.C.; software, M.C. and E.C.; validation, M.C., E.C. and A.L.; formal analysis, M.C. and E.C.; investigation, M.C. and E.C.; resources, M.C. and A.L.; data curation, M.C., E.C. and A.L.; writing—original draft preparation, M.C., E.C. and A.L.; writing—review and editing, M.C., E.C., D.M. and A.L.; supervision, A.L.; funding acquisition, A.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the University of Padova under prot. DOR 2032990.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The authors declare that the data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format, they can be made available by the corresponding author upon reasonable request.

Acknowledgments: The authors thank Stefania Zannoni, the Distillerie Bonollo (Conselve, Italy) and the "Associazione Nazionale Industriale Distillatori di Alcoli e Acquaviti" for their technical support.

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

Abbreviations

AAI, Antioxidant Activity Index; AOA, antioxidant activity; BBD, Box–Behnken Design; BHT, Butylhydroxytoluene; EGPs, extracts from grape pomace; FRAP, ferric reducing antioxidant potential; GAE, Gallic Acid Equivalent; GP, grape pomace; IT, Induction Time; RP, red pomace; RSM, Response Surface Methodology; SGP, spent grape pomace; TE, Trolox Equivalent; TPC, total phenolic content; TPTZ, 2,3,5–Triphenyltetrazolium; Trolox, Tetramethylchromane–2–carboxylic acid; UAE, ultrasound-assisted extraction; WB, water bath; WP, white pomace; WUS, water bath + ultrasound.

References

1. Wiśniewska, P.; Śliwińska, M.; Dymerski, T.; Wardencki, W.; Namieśnik, J. The analysis of raw spirits—A review of methodology. *J. Inst. Brew.* **2016**, *122*, 5–10. [CrossRef]
2. Da Porto, C. Grappa: Production, sensory properties and market development. In *Alcoholic Beverages*; Woodhead Publishing: Camvridge, UK, 2012; pp. 299–314.
3. Gazzetta Ufficiale della Repubblica Italiana. DPR n.297. 1997. Available online: https://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=1997-09-12&atto.codiceRedazionale=097G0340 (accessed on 3 September 2024).

4. Gazzetta Ufficiale dell'Unione Europea. Regolamento (UE) 787. 2019. Available online: https://eur-lex.europa.eu/legal-content/IT/TXT/PDF/?uri=CELEX:32019R0787&from=EN (accessed on 3 September 2024).
5. Cisneros-Yupanqui, M.; Lante, A.; Mihaylova, D.; Krastanov, A.I.; Rizzi, C. The α-Amylase and α-Glucosidase Inhibition Capacity of Grape Pomace: A Review. *Food Bioprocess Technol.* **2023**, *16*, 691–703. [CrossRef] [PubMed]
6. Mayr Marangon, C.; De Rosso, M.; Carraro, R.; Flamini, R. Changes in volatile compounds of grape pomace distillate (Italian *grappa*) during one-year ageing in oak and cherry barrels. *Food Chem.* **2021**, *344*, 128658. [CrossRef]
7. Poli Museo della Grappa. Grappa.com. 2023. La Materia Prima: La Vinaccia. Available online: https://www.grappa.com/ita/index.php (accessed on 5 June 2023).
8. AssoDistil. *AssoDistil Annual Report 2023*; AssoDistil: Rome, Italy, 2023.
9. Bordiga, M.; Travaglia, F.; Locatelli, M.; Arlorio, M.; Coïsson, J.D. Spent grape pomace as a still potential by-product. *Int. J. Food Sci. Technol.* **2015**, *50*, 2022–2031. [CrossRef]
10. Pagano, I.; Campone, L.; Celano, R.; Piccinelli, A.L.; Rastrelli, L. Green non-conventional techniques for the extraction of polyphenols from agricultural food by-products: A review. *J. Chromatogr. A* **2021**, *1651*, 462295. [CrossRef]
11. Picot-Allain, C.; Mahomoodally, M.F.; Ak, G.; Zengin, G. Conventional versus green extraction techniques—A comparative perspective. *Curr. Opin. Food Sci.* **2021**, *40*, 144–156. [CrossRef]
12. Mihaylova, D.; Lante, A. Water an Eco-Friendly Crossroad in Green Extraction: An Overview. *Open Biotechnol. J.* **2019**, *13*, 155–162. [CrossRef]
13. More, P.R.; Jambrak, A.R.; Arya, S.S. Green, environment-friendly and sustainable techniques for extraction of food bioactive compounds and waste valorization. *Trends Food Sci. Technol.* **2022**, *128*, 296–315. [CrossRef]
14. Ozsefil, I.C.; Ziylan-Yavas, A. Green approach for polyphenol extraction from waste tea biomass: Single and hybrid application of conventional and ultrasound-assisted extraction. *Environ. Res.* **2023**, *235*, 116703. [CrossRef]
15. Cisneros-Yupanqui, M.; Zagotto, A.; Alberton, A.; Lante, A.; Zagotto, G.; Ribaudo, G.; Rizzi, C. Monitoring the antioxidant activity of an eco-friendly processed grape pomace along the storage. *Nat. Prod. Res.* **2021**, *35*, 6030–6033. [CrossRef]
16. 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. Available online: https://journals.sagepub.com/doi/10.4137/NMI.S32910 (accessed on 8 November 2022). [CrossRef] [PubMed]
17. Campos, D.; García-Ríos, D.; Aguilar-Galvez, A.; Chirinos, R.; Pedreschi, R. Comparison of conventional and ultrasound-assisted extractions of polyphenols from Inca muña (*Clinopodium bolivianum*) and their characterization using UPLC–PDA–ESI–Q/TOF–MSn technique. *J. Food Process. Preserv.* **2022**, *46*, e16310. [CrossRef]
18. Cisneros-Yupanqui, M.; Rizzi, C.; Mihaylova, D.; Lante, A. Effect of the distillation process on polyphenols content of grape pomace. *Eur. Food Res. Technol.* **2021**, *248*, 929–935. [CrossRef]
19. Codex Alimentarius. "Codex General Standard for Food Additives". (GSFA, Codex STAN 192-1995). 2023. Available online: https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/gsfa/en/ (accessed on 29 September 2024).
20. Da Rocha, C.B.; Noreña, C.P.Z. Microwave-Assisted Extraction and Ultrasound-Assisted Extraction of Bioactive Compounds from Grape Pomace. *Int. J. Food Eng.* **2020**, *16*, 20190191. [CrossRef]
21. Dzah, C.S.; Duan, Y.; Zhang, H.; Wen, C.; Zhang, J.; Chen, G.; Ma, H. The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. *Food Biosci.* **2020**, *35*, 100547. [CrossRef]
22. Ebrahimi, P.; Bayram, I.; Mihaylova, D.; Lante, A. A Strategy to Minimize the Chlorophyll Content in the Phenolic Extract of Sugar Beet Leaves: Can this Extract Work as a Natural Antioxidant in Vegetable Oils? *Food Bioprocess Technol.* **2024**. [CrossRef]
23. Silva Júnior, M.E.; Araújo, M.V.R.L.; Santana, A.A.; Silva, F.L.H.; Maciel, M.I.S. Ultrasound-assisted extraction of bioactive compounds from ciriguela (*Spondias purpurea* L.) peel: Optimization and comparison with conventional extraction and microwave. *Arab. J. Chem.* **2021**, *14*, 103260. [CrossRef]
24. Castañeda-Valbuena, D.; Ayora-Talavera, T.; Luján-Hidalgo, C.; Álvarez-Gutiérrez, P.; Martínez-Galero, N.; Meza-Gordillo, R. Ultrasound extraction conditions effect on antioxidant capacity of mango by-product extracts. *Food Bioprod. Process.* **2021**, *127*, 212–224. [CrossRef]
25. Berkani, F.; Serralheiro, M.L.; Dahmoune, F.; Ressaissi, A.; Kadri, N.; Remini, H. Ultrasound assisted extraction of phenolic compounds from a jujube by-product with valuable bioactivities. *Processes* **2020**, *8*, 1441. [CrossRef]
26. Medina-Torres, N.; Ayora-Talavera, T.; Espinosa-Andrews, H.; Sánchez-Contreras, A.; Pacheco, N. Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources. *Agronomy* **2017**, *7*, 47. [CrossRef]
27. Antony, A.; Farid, M. Effect of Temperatures on Polyphenols during Extraction. *Appl. Sci.* **2022**, *12*, 2107. [CrossRef]
28. González-Centeno, M.R.; Comas-Serra, F.; Femenia, A.; Rosselló, C.; Simal, S. Effect of power ultrasound application on aqueous extraction of phenolic compounds and antioxidant capacity from grape pomace (*Vitis vinifera* L.): Experimental kinetics and modeling. *Ultrason. Sonochem.* **2015**, *22*, 506–514. [CrossRef] [PubMed]
29. Caldas, T.W.; Mazza, K.E.L.; Teles, A.S.C.; Mattos, G.N.; Brígida, A.I.S.; Conte-Junior, C.A.; Borguini, R.G.; Godoy, R.L.O.; Cabral, L.M.C.; Tonon, R.V. Phenolic compounds recovery from grape skin using conventional and non-conventional extraction methods. *Ind. Crops Prod.* **2018**, *111*, 86–91. [CrossRef]
30. Christou, A.; Stavrou, I.J.; Kapnissi-Christodoulou, C.P. Continuous and pulsed ultrasound-assisted extraction of carob's antioxidants: Processing parameters optimization and identification of polyphenolic composition. *Ultrason. Sonochem.* **2021**, *76*, 105630. [CrossRef] [PubMed]

31. Fontana, A.R.; Antoniolli, A.; Bottini, R. Grape pomace as a sustainable source of bioactive compounds: Extraction, characterization, and biotechnological applications of phenolics. *J. Agric. Food Chem.* **2013**, *61*, 8987–9003. [CrossRef]
32. Casagrande, M.; Zanela, J.; Pereira, D.; de Lima, V.A.; Oldoni, T.L.C.; Carpes, S.T. Optimization of the extraction of antioxidant phenolic compounds from grape pomace using response surface methodology. *J. Food Meas. Charact.* **2019**, *13*, 1120–1129. [CrossRef]
33. Rodrigues, R.P.; Sousa, A.M.; Gando-Ferreira, L.M.; Quina, M.J. Grape Pomace as a Natural Source of Phenolic Compounds: Solvent Screening and Extraction Optimization. *Molecules* **2023**, *28*, 2715. [CrossRef]
34. Pintać, D.; Majkić, T.; Torović, L.; Orčić, D.; Beara, I.; Simin, N.; Mimica–Dukić, N.; Lesjak, M. Solvent selection for efficient extraction of bioactive compounds from grape pomace. *Ind. Crops Prod.* **2018**, *111*, 379–390. [CrossRef]
35. Tournour, H.H.; Segundo, M.A.; Magalhães, L.M.; Barreiros, L.; Queiroz, J.; Cunha, L.M. Valorization of grape pomace: Extraction of bioactive phenolics with antioxidant properties. *Ind. Crops Prod.* **2015**, *74*, 397–406. [CrossRef]
36. Chemat, F.; Vian, M.A.; Cravotto, G. Green extraction of natural products: Concept and principles. *Int. J. Mol. Sci.* **2012**, *13*, 8615–8627. [CrossRef]
37. Scharlack, N.K.; Aracava, K.K.; Rodrigues, C.E.C. Effect of the type and level of hydration of alcoholic solvents on the simultaneous extraction of oil and chlorogenic acids from sunflower seed press cake. *J. Sci. Food Agric.* **2017**, *97*, 4612–4620. [CrossRef]
38. Kashyap, P.; Singh, C.; Navdeep, R. Optimization of ultrasound assisted extraction of polyphenols from Meghalayan cherry fruit (*Prunus nepalensis*) using response surface methodology (RSM) and artificial neural network (ANN) approach. *J. Food Meas. Charact.* **2021**, *15*, 119–133. [CrossRef]
39. Bouaoudia-Madi, N.; Boulekbache-Makhlouf, L.; Madani, K.; Silva, A.M.S.; Dairi, S.; Oukhmano-Bensidhoum, S.; Cardoso, S.M. Optimization of ultrasound-assisted extraction of polyphenols from *Myrtus communis* L. Pericarp. *Antioxidants* **2019**, *8*, 205. [CrossRef]
40. Muñoz-Bernal, Ó.A.; Coria-Oliveros, A.J.; de la Rosa, L.A.; Rodrigo-García, J.; del Rocío Martínez-Ruiz, N.; Sayago-Ayerdi, S.G.; Alvarez-Parrilla, E. Cardioprotective effect of red wine and grape pomace. *Food Res. Int.* **2021**, *140*, 110069. [CrossRef]
41. Cisneros-Yupanqui, M.; Chalova, V.I.; Kalaydzhiev, H.R.; Mihaylova, D.; Krastanov, A.I.; Lante, A. Ultrasound-assisted extraction of antioxidant bioactive compounds from wastes of rapeseed industry and their application in delaying rapeseed oil oxidation. *Environ. Technol. Innov.* **2023**, *30*, 103081. [CrossRef]
42. Sousa, G.; Trifunovska, M.; Antunes, M.; Miranda, I.; Mold, M.; Alves, V.; Vidrih, R.; Lopes, P.A.; Aparicio, L.; Neves, M.; et al. Optimization of Ultrasound-Assisted Extraction of Bioactive Compounds from *Pelvetia canaliculata* to Sunflower Oil. *Foods* **2021**, *10*, 1732. [CrossRef]
43. Haaland, P.D. *Experimental Design in Biotechnology*; CRC Press: Boca Raton, FL, USA, 1989.
44. Rebollo-Hernanz, M.; Cañas, S.; Taladrid, D.; Segovia, Á.; Bartolomé, B.; Aguilera, Y.; Martín-Cabrejas, M.A. Extraction of phenolic compounds from cocoa shell: Modeling using response surface methodology and artificial neural networks. *Sep. Purif. Technol.* **2021**, *270*, 118779. [CrossRef]
45. Ojeda, G.A.; Sgroppo, S.C.; Sánchez Moreno, C.; de Ancos Siguero, B. Mango 'criollo' by-products as a source of polyphenols with antioxidant capacity. Ultrasound assisted extraction evaluated by response surface methodology and HPLC-ESI-QTOF-MS/MS characterization. *Food Chem.* **2022**, *396*, 133738. [CrossRef]
46. Moradi, D.; Ramezan, Y.; Eskandari, S.; Mirsaeedghazi, H.; Dakheli, M.J. Optimization of polyphenol recovery from potato peel and its incorporation into low-density polyethylene films activated by cold plasma. *Biomass Convers. Biorefin.* **2024**, *14*, 14209–14223. [CrossRef]
47. Hossain, M.B.; Brunton, N.P.; Patras, A.; Tiwari, B.; O'Donnell, C.P.; Martin-Diana, A.B.; Barry-Ryan, C. Optimization of ultrasound assisted extraction of antioxidant compounds from marjoram (*Origanum majorana* L.) using response surface methodology. *Ultrason. Sonochem.* **2012**, *19*, 582–590. [CrossRef]
48. Kaur, S.; Panesar, P.S.; Chopra, H.K. Standardization of ultrasound-assisted extraction of bioactive compounds from kinnow mandarin peel. *Biomass Convers. Biorefin.* **2023**, *13*, 8853–8863. [CrossRef]
49. Osorio-Tobón, J.F. Recent advances and comparisons of conventional and alternative extraction techniques of phenolic compounds. *J. Food Sci. Technol.* **2020**, *57*, 4299–4315. [CrossRef] [PubMed]
50. Rodsamran, P.; Sothornvit, R. Extraction of phenolic compounds from lime peel waste using ultrasonic-assisted and microwave-assisted extractions. *Food Biosci.* **2019**, *28*, 66–73. [CrossRef]
51. Palsikowski, P.A.; Besen, L.M.; Klein, E.J.; da Silva, C.; da Silva, E.A. Optimization of ultrasound-assisted extraction of bioactive compounds from *B. forficata* subsp. *Pruinosa*. *Can. J. Chem. Eng.* **2020**, *98*, 2214–2226. [CrossRef]
52. Babaoğlu, A.S.; Dilek, N.M.; Karakaya, M.; Unal, K. Valorization of sugar beet molasses powder by microwave and ultrasound-assisted extractions of bioactive compounds: An optimization study. *J. Food Process. Preserv.* **2022**, *46*, e16820. [CrossRef]
53. Chavan, Y.; Singhal, R.S. Ultrasound-assisted extraction (UAE) of bioactives from arecanut (*Areca catechu* L.) and optimization study using response surface methodology. *Innov. Food Sci. Emerg. Technol.* **2013**, *17*, 106–113. [CrossRef]
54. Foujdar, R.; Bera, M.B.; Chopra, H.K. Optimization of process variables of probe ultrasonic-assisted extraction of phenolic compounds from the peel of *Punica granatum* Var. Bhagwa and it's chemical and bioactivity characterization. *J. Food Process. Preserv.* **2020**, *44*, e14317. [CrossRef]
55. Clodoveo, M.L.; Crupi, P.; Corbo, F. Optimization of a Green Extraction of Polyphenols from Sweet Cherry (*Prunus avium* L.) Pulp. *Processes* **2022**, *10*, 1657. [CrossRef]

56. Ranjha, M.M.A.N.; Irfan, S.; Lorenzo, J.M.; Shafique, B.; Kanwal, R.; Pateiro, M.; Arshad, R.N.; Wang, L.; Nayik, G.A.; Roobab, U.; et al. Sonication, a potential technique for extraction of phytoconstituents: A systematic review. *Processes* **2021**, *9*, 1406. [CrossRef]
57. Ahmed, T.; Rana, M.R.; Hossain, M.A.; Ullah, S.; Suzauddula, M. Optimization of Ultrasound-Assisted Extraction Using Response Surface Methodology for Total Anthocyanin Content, Total Phenolic Content, and Antioxidant Activities of Roselle (*Hibiscus sabdariffa* L.) Calyces and Comparison with Conventional Soxhlet Extraction. *Biomass Convers. Biorefin.* **2023**. [CrossRef]
58. Hu, D.B.; Xue, R.; Zhuang, X.C.; Zhang, X.S.; Shi, S.L. Ultrasound-assisted extraction optimization of polyphenols from *Boletus bicolor* and evaluation of its antioxidant activity. *Front. Nutr.* **2023**, *10*, 1135712. [CrossRef] [PubMed]
59. Fajardo Daza, J.A.; Ibarra, C.A.; Arturo Perdomo, D.; Herrera Ruales, F.C. Optimization of ultrasound assisted extraction of polyphenols in cocoa beans. *Vitae* **2020**, *27*, 1–8. [CrossRef]
60. Juliana, D.; Aisyah, S.I.; Priosoeryanto, B.P.; Nurcholis, W. Optimization of cardamom (*Amomum compactum*) fruit extraction using the Box—Behnken design focused on polyphenol extraction with antioxidant activity. *Appl. Pharm. Sci.* **2022**, *12*, 194–209. [CrossRef]
61. Dairi, S.; Carbonneau, M.A.; Galeano-Diaz, T.; Remini, H.; Dahmoune, F.; Aoun, O.; Belbahi, A.; Lauret, C.; Cristol, J.P.; Madani, K. Antioxidant effects of extra virgin olive oil enriched by myrtle phenolic extracts on iron-mediated lipid peroxidation under intestinal conditions model. *Food Chem.* **2017**, *237*, 297–304. [CrossRef]
62. Blasi, F.; Cossignani, L. An overview of natural extracts with antioxidant activity for the improvement of the oxidative stability and shelf life of edible oils. *Processes* **2020**, *8*, 956. [CrossRef]
63. Montesano, D.; Rocchetti, G.; Cossignani, L.; Senizza, B.; Pollini, L.; Lucini, L.; Blasi, F. Untargeted Metabolomics to Evaluate the Stability of Extra-Virgin Olive Oil with Added *Lycium barbarum* Carotenoids during Storage. *Foods* **2019**, *8*, 179. [CrossRef]
64. Nhat, N.; Phuong, M.; Le, T.T.; Viet, M.; Nguyen, T.; Camp, J.V.; Raes, K. Antioxidant Activity of Rambutan (*Nephelium lappaceum* L.) Peel Extract in Soybean Oil during Storage and Deep Frying. *Eur. J. Lipid Sci. Technol.* **2020**, *122*, 1900214.
65. Sordini, B.; Veneziani, G.; Servili, M.; Esposto, S.; Selvaggini, R.; Lorefice, A.; Taticchi, A. A quanti-qualitative study of a phenolic extract as a natural antioxidant in the frying processes. *Food Chem.* **2019**, *279*, 426–434. [CrossRef]
66. Rahimi, S.; Mikani, M. Lycopene green ultrasound-assisted extraction using edible oil accompany with response surface methodology (RSM) optimization performance: Application in tomato processing wastes. *Microchem. J.* **2019**, *146*, 1033–1042. [CrossRef]
67. Cisneros-Yupanqui, M.; Chalova, V.I.; Kalaydzhiev, H.R.; Mihaylova, D.; Krastanov, A.I.; Lante, A. Preliminary Characterisation of Wastes Generated from the Rapeseed and Sunflower Protein Isolation Process and Their Valorisation in Delaying Oil Oxidation. *Food Bioprocess Technol.* **2021**, *14*, 1962–1971. [CrossRef]
68. Zacometti, C.; Lante, A.; Cisneros, M.; Massaro, A.; Mihaylova, D.; Chalova, V.; Krastanov, A.; Kalaydzhiev, H.; Riuzzi, G.; Tata, A.; et al. Rapid Assessment of Metabolomic Fingerprinting of Recycled Sunflower By-Products via DART–HRMS. *Molecules* **2024**, *29*, 4092. [CrossRef] [PubMed]
69. Szydłowska-Czerniak, A.; Amarowicz, R.; Szłyk, E. Antioxidant capacity of rapeseed meal and rapeseed oils enriched with meal extract. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 750–760. [CrossRef]
70. Bayram, I.; Decker, E.A. Underlying mechanisms of synergistic antioxidant interactions during lipid oxidation. *Trends Food Sci. Technol.* **2023**, *133*, 219–230. [CrossRef]