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Bioactivity of Grape Skin from Small-Berry Muscat and Augustiatis of Samos: A Circular Economy Perspective for Sustainability

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Abstract: Consumer interest in health-promoting foods has prompted researchers to use wine by-products to increase food's functional characteristics. This research aims to examine the skin bioactivities of Samos white (small-berry Muscat) and red (Augustiatis) grape skin extracts (M-GSkE, A-GSkE). Total phenolic content, antiradical activity, the inhibition of plasma oxidation and platelet aggregation, and the phenolic profile were examined. A-GSkE and M-GSkE showed high total phenolics (1.19 ± 0.13 vs. 2.12 ± 0.23 mM GAE), antiradical activity (7.7 ± 0.4 vs. 6.6 ± 0.3 μM GAE for ABTS; 31.12 ± 0.8 vs. 26.4 ± 1.0 μM GAE for DPPH), resistance to plasma oxidation (5.7 ± 0.4 vs. 1.1 ± 0.2 μM GAE), and antithrombotic activity (19.7 ± 0.1 vs. 26.6 ± 0.2 μM GAE). Ferulic (41.3 ± 0.1 > 13.2 ± 0.1 μg/g DM), vanillic (26.3 ± 1.7 > 12.2 ± 1.2 μg/g DM), and gallic (16.6 ± 0.1 > 8.4 ± 2.9 μg/g DM) acids along with ε-viniferin (3.6 ± 0.4 > 2.8 ± 0.3 μg/g DM) were identified in higher content in A-GSkE. Catechin (59.8 ± 1.5 μg/g DM), chlorogenic acid (43.8 ± 0.9 μg/g DM), and resveratrol (0.83 ± 0.13 μg/g DM) were identified only in M-GSkE, while caffeic acid 19.8 ± 0.4 μg/g DM) and daidzein (16.8 ± 0.1 μg/g DM) were identified only in A-GSkE. The specialized bioactivities researched in two previously unexplored Samos' wine grape skin extracts give them added value. The valorization of such by-products promises a sustainable future in the food sector of local communities and an improvement in local public health.

Keywords: grape skin; wine making by-products; phenolics; antiradical activity; antioxidant activity; antiplatelet activity

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1. Introduction

Because of the significant quantity of by-products generated in the food sector, economic and environmental issues have escalated in recent decades. One of the major concerns in this area involves the redefining of such by-products as raw materials that may be processed, with the objective of limiting their negative environment impacts while gaining high value-added foods. This action could lead in the future to a more sustainable food sector.

The wine industry produces a lot of by-products, which are usually utilized as organic fertilizer or animal feed. Moreover, viticulture has seen tremendous expansion in the last decade [1], and wine production, along with its by-products, have been increased dramatically.

Viticulture plays a vital role in the European economy. Greece ranks seventh in the EU [2] in terms of grape volume produced. The result of the processing of these grapes during wine production is a substantial quantity of grape skin in the form of grape pomace.

Alternative uses for wine industry by-products are being researched, having in mind factors such as environmental benefits, financial savings, and new potential for industrial development. Recent studies have suggested that nonextracted items, such as grape skin bioactive chemicals, may be of significant interest [3–5].

The demand for functional foods has been increasing, and one solution that might be considered for satisfying this need is the use of by-products, such as the grape skin derived from grape pomace during wine production [6–8].

Applying this reasoning to areas with many small islands, such as that of the northern Aegean in Greece, the goal of promoting innovation and entrepreneurship in the context of the agri-food sector is served.

The exploitation of the grape skin by-product, which is abundant in these islands, could lead to the production of new functional foods or the further development of already existing traditional products. Increased consumer demand for such foods will in turn lead to more sustainable conditions in the future for local communities in these areas.

Grape skin has been recognized for its large quantities of bioactive compounds [9,10] that add value to these by-products owing to their various potential applications in the food and pharmaceutical sectors [11,12]. Previous studies have referred to the antioxidant, antibacterial, anti-inflammatory, antiobesity, and anticancer properties of the biomolecules that are obtained from grape skin [3–5,13–17].

Other studies have shown that the oxidation of plasma lipoproteins has been demonstrated to initiate atherosclerosis at the molecular level. This oxidation procedure results in the production of thrombotic and inflammatory lipid mediators such as platelet-activating factor (PAF) and PAF-like oxidized phospholipids, which mediate the early stages of inflammation on the aortic endothelium as well as thrombosis and free radical production [18–20]. In addition, minor bioactive substances in plant-originating foods that exhibit antioxidant and/or PAF-inhibitory effects have been shown in studies to be important in the prevention of cardiovascular disease [18,21].

The aim of this study was to highlight the bioactivities of grape skins from two unexplored winemaking grape varieties that are cultivated in the Greek island of Samos in Northern Aegean from the perspective of the nutraceutical value related to antiatherogenic activities such as free radical scavenging and the inhibition of plasma oxidation and PAF-induced platelet aggregation. Total phenolic content (TPC), radical scavenging, and the inhibition of platelet aggregation and plasma oxidation were evaluated in grape skin methanolic extracts.

2. Materials and Methods

2.1. Chemicals and Reagents

Folin–Ciocalteu and di-sodium hydrogen phosphate dihydrate was supplied by Merck (Darmstadt, Germany). Anhydrous sodium carbonate was purchased from SDS (Peypin, France). The reagents of gallic acid; 1,1-Diphenyl-2-picryl-hydrazyl (DPPH); fatty acid-free bovine serum albumin, beta-acetyl-O-hexadecyl-L-phosphatidylcholine (PAF), as well as formic acid and solvents of methanol and water for HPLC analysis, were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Trolox was supplied by Acros Organics (Waltham, MA, USA). Sodium chloride and sodium dihydrogen phosphate dehydrate were purchased from Penta (CZ Ltd., Chrudim, Czech Republic). The 2,2′-Azino-bis(3-ethylbezothiazoline-6-sulphonic acid (ABTS) reagent was acquired from Applichem (Darmstadt, Germany). Chem-Lab was the supplier of potassium persulfate (Zedelgem, Belgium). The purchase of copper sulphate pentahydrate was from Alfa Aesar (Ward Hill, MA, USA).

2.2. Material for Analysis

2.2.1. Preparation of Grape Skin Samples

Grapes cultivated in the Greek island of Samos from small-berry Muscat and Augustiatis were harvested at technological maturation in August 2020. The grapes were then pressed to obtain the juice for the production of wine. The by-product of the processing

consisted of grape skin, grape seeds, and grape stems and was used to manually obtain the grape skin samples. To reduce the humidity of the samples, grape skins were stored in a deep freeze (DW-HL388, Zhongke Meiling Cryogenics corp., Hefei, China) at −86 °C for 1 day and then lyophilized for 48 h under vacuum (5.0 Pascal) at −60 °C using a freeze dryer BK-FD10PT (Biobase Biodustry Co., Ltd., Jinan, China). The dry grape skin samples were then processed for one minute in a laboratory grinder IKA A 10 basic (IKA Works, Wilmington, NC, USA) to produce a sample of fine powder.

2.2.2. Extraction of Grape Skin Samples

The selection of the solvent for the extraction of phenolics from the grape skin samples was based on an analysis which utilized three distinct methanol/water solvent mixtures with ratios 80/20, 70/30, and 60/40 (v/v) and a ratio of (solvent mixture)/(grape skin sample) equal to 100 (v/w). The grape skin samples with the various solvent mixtures were placed in polystyrene test tubes with a stopper and extracted through pulsed ultrasound assistance at 37 kHz and 220 W ultrasonic power using an elmasonic P 70 H ultrasonic device (Elmasonic P; Elma, Singen, Germany) at 50 °C for 30 min. The samples were then stored at −86 °C for 24 h, followed by centrifugation at 20 °C for 15 min at 20,000× g. Until further analysis, the supernatant was aliquoted in 2.0 mL portions and kept in polypropylene microvials at −86 °C. The extract with the methanol/water ratio that resulted in the strongest antiradical activity based on the ABTS assay was adopted for further study of the samples.

2.3. Determination of Phenolic Compounds

The total phenolic content was measured in triplicate in grape skin methanolic extracts using a modified version of Singleton and Rossi's technique [22]. The experiment was carried out by combining 0.01 to 0.001 mL of extract with 1.8 mL of distilled water and 0.1 mL of Folin–Ciocalteu reagent. The materials were then rapidly mixed and incubated in the dark for two minutes. After adding 0.3 mL of 20% (w/v) aqueous Na2CO3, the samples were rapidly agitated and incubated at 40 °C in a water bath for 30 min. Absorbance was measured spectrophotometrically at 765 nm using a Spectrophotometer Lambda 25 (Perkin Elmer, Norwalk, CT, USA). Gallic acid was used to develop a standard curve. The final findings were expressed as equivalent concentrations of gallic acid (mM GAE).

2.4. Radical Scavenging Properties Evaluation

2.4.1. ABTS Assay

The ABTS radical scavenging activity of extracts was determined using a modified version of the technique of Re et al. [23]. The $ABTS^{+\bullet}$ was generated by reacting a 7 mmol/L stock solution of ABTS with a 2.45 mmol/L final concentration of potassium persulphate ($K_2S_2O_8$). The $ABTS^{+\bullet}$ solution was diluted to an absorbance of 0.700 ± 0.050 at 734 nm with distilled water. Aliquots of grape skin extracts or suitable volumes of Trolox, as positive reference compound, were combined with 1.0 mL $ABTS^{+\bullet}$. The absorbance at 734 nm was determined spectrophotometrically after vigorous stirring and a 15-min incubation of samples in the dark at room temperature. The capacity of the extracts to scavenge the ABTS free cationic radical was examined compared to a control sample containing distilled water instead of each amount of the extracts tested. The results were expressed as concentration of GAE in μM able for 50% scavenging of $ABTS^{+\bullet}$ ($IC_{50\text{-}ABTS}$) and as a Trolox-equivalent amount. Each sample was evaluated in triplicate.

2.4.2. DPPH Assay

The ability of grape skin extracts to scavenge the DPPH free radical was evaluated using a modified version of Abe, Murata, and Hirota's technique [24]. An aliquot of the extracts or a suitable standard solution of Trolox, as a positive reference compound, was diluted to a volume of 0.9 mL with methanol. Then, 0.1 mL of DPPH reagent in methanol at a concentration of 6.0 mM was added, followed by vigorous stirring. After 15 min in the

dark, the absorbance was measured spectrophotometrically at 515 nm against a reference sample containing methanol in place of each volume of grape skin extracts examined. The findings were presented as concentration of GAE in μM able for 50% scavenging of DPPH ($IC_{50\text{-DPPH}}$) and as a Trolox-equivalent amount. Each sample was examined in triplicate.

2.5. Plasma Oxidation

The human plasma oxidation inhibition experiment was performed according to Schnitzer et al. [25] with minimal modifications utilizing a Lambda 25 Spectrophotometer (Perkin–Elmer, Norwalk, CT, USA) equipped with an eight-position thermostatic sample changer. Grape skin extracts were deposited in UV-transparent disposable cuvettes (Brand, Wertheim, Germany). Then, 880 μL of phosphate buffer solution (PBS), pH 7.4, 146 mM in NaCl, and 20 μL of human plasma were added. After moderate shaking and incubation at room temperature for 1 min, the samples were placed in the photometer's thermostatic chamber and incubated for 10 min at 37 °C. The oxidation process was initiated by adding 100 μL of 1 mM $CuSO_4{\cdot}5H_2O$. The absorbance was measured continuously for 3.0 h at 245 nm and at a constant temperature of 37 °C. The duration of plasma's resistance to oxidation in the presence of grape skin extracts, PBS, or Trolox was assessed by the absence of a rise in absorbance at 245 nm. The prevention of in vitro plasma oxidation generated by the extracts was assessed by comparing the plasma oxidation resistance time of each sample containing the extract to that of a reference sample containing PBS instead of each tested volume of grape skin sample. Trolox was used as a positive reference compound. The findings were represented as concentration of GAE in μM able to produce a 50% increase in plasma oxidation lag time ($LTIC_{50\text{-POX}}$) and as a Trolox-equivalent amount.

2.6. Platelet Aggregation Assay

Grape skin extracts were evaluated for their in vitro antithrombotic effectiveness using the PAF-induced thrombosis inhibition assay in platelet-rich plasma (PRP). The experiment was performed using a Chrono-Log 500-Ca aggregometer (Chrono-Log Co., Havertown, PA, USA) linked to a computer (Aggro/Link software; Chrono-Log, Hawerstown, PA, USA) [26]. Under a stream of nitrogen, aliquots of grape skin extracts and PAF ethanolic solution were evaporated and reconstituted in bovine serum albumin (BSA) (2.5 mg/mL saline). Then, 250 μL aliquots of PRP and stir bars were placed in siliconized glass cuvettes and incubated for 15 min at 37 °C in the incubation wells of the aggregometer. Next, the platelet response caused by a final concentration of 0.27 μM PAF in PRP was measured. The resulting curves were recorded before (assumed to be 0% inhibition) and after the addition of different quantities of the studied extracts in the presence of PAF while stirring at 1200 rpm. The quantity of extracts necessary to inhibit PAF activity by 50 percent was estimated utilizing the area of 20 to 80 percent inhibition against varying quantities of grape skin extracts and was expressed as concentration of GAE in μM capable of producing 50% inhibition of the PAF-induced PRP aggregation ($IC_{50\text{-PAF}}$).

2.7. HPLC-DAD Analysis of Phenolics in Grape Skin Extracts

For the analysis of phenolic compounds in grape skin extracts, a Shimadzu LC-2030 C prominence-i system equipped with a binary pump, degasser, autosampler, column heater, and PDA detector was used (Shimadzu, Kyoto, Japan). For the separation of the phenolic compounds under analysis, the analytical column of Luna C18(2), 5 μm, 4.6 mm × 250 mm from Phenomenex (Aschaffenburg, Germany) was used. The elution was carried out using 0.2% (v/v) formic acid-acidified water (mobile phase A) and methanol (mobile phase B). The following is how the chosen elution gradient scheme was implemented: 5% mobile phase B at 0 min; 5% mobile phase B at 2 min; 95% mobile phase B at 20 min; 95% mobile phase B at 25 min; 5% mobile phase B at 25.01 min; 5% mobile phase B at 28 min. The volume of injection was 20 μL. UV–vis spectra were recorded between 190 and 800 nm, and chromatograms were obtained at 280 nm. Gallic acid, ferulic acid, vanillic acid, daidzein, chlorogenic acid, caffeic acid, (+)-catechin, protocatechic acid, tyrosol, resveratrol, and viniferin were employed as standards.

2.8. Statistical Analysis

Data are presented as mean ± standard deviation (SD) after statistical analysis by ANOVA with Tukey's post hoc, Student's *t*-test, and Pearson's correlation analysis using SPSS (version 28.0; SPSS Inc., Chicago, IL, USA).

3. Results

The study of grape skin methanolic extracts from small-berry Muscat and Augustiatis cultivated in the Greek island of Samos were carried out by estimating the following parameters: (1) extractive capacity of antioxidants by aqueous methanol solutions, (2) total phenolic determination and radical scavenging activity, (3) antiplatelet and plasma antioxidant activities, and (4) free phenolic profile by HPLC-DAD analysis.

3.1. Extractive Capacity of Antioxidants by Aqueous Methanol Solutions

The extractive capacity of antioxidants by aqueous methanol solutions was evaluated using ABTS radical cation scavenging activity. The results, as assessed by the ABTS assay, are presented in Table 1. Grape skin extracts from both samples exerted higher radical scavenging activity when extracted through methanol/water 60/40 (*v*/*v*). For this reason, the ratio of 60/40 for methanol/water was adopted as the solvent to produce extracts for further study.

Table 1. ABTS radical cation scavenging amount of grape skin extracts obtained from different solvent mixtures.

	[1]M-GSkE	[2]A-GSkE
Solvent mixture	[3]$SA_{50\text{-ABTS}}$ (μL)	$SA_{50\text{-ABTS}}$ (μL)
[4]M/W: 80/20 (*v*/*v*)	10.3 ± 0.4 [a]	4.9 ± 0.3 [b]
M/W: 70/30 (*v*/*v*)	9.8 ± 0.3 [a]	4.7 ± 0.2 [b]
M/W: 60/40 (*v*/*v*)	6.5 ± 0.3 [c]	3.1 ± 0.1 [d]

[1]M-GSkE: small-berry Muscat grape skin extract; [2]A-GSkE: Augustiatis grape skin extract; [3]SA_{50}: Amount for 50% scavenging of the ABTS radical cation; [4]M/W: Methanol/Water. Different letters (a–d) in rows and columns denote values of statistically significant difference. Results are expressed as mean ± SD in microliters of extract. Extraction was performed using 100 mL of solvent per 1 g of dry powder matter of each grape skin sample.

3.2. Total Phenolic Content and Radical Scavenging Activity Determination

The TPC of grape skin methanolic extracts are reported in Table 2 as mM of gallic acid equivalent (GAE). The total phenolic content in Augustiatis grape skin extract (A-GSkE) was higher than that from the small-berry Muscat grape skin extract (M-GSkE) (2.12 ± 0.23 > 1.19 ± 0.13 mM GAE; $p < 0.05$). The radical scavenging activity of the two extracts, as evaluated by the ABTS and DPPH assays, was expressed as μM GAE and showed higher antiradical activity for A-GSkE compared to M-GSkE ($p < 0.05$).

Table 2. Total phenolic content and radical scavenging activities.

Parameters	[1]M-GSkE	[2]A-GSkE
[3]TPC (mM GAE)	1.19 ± 0.13 [a]	2.12 ± 0.23
[4]$IC_{50\text{-ABTS}}$ (μM GAE)	7.7 ± 0.4 [a]	6.6 ± 0.3
$IC_{50\text{-DPPH}}$ (μM GAE)	31.2 ± 0.8 [a]	26.4 ± 1.0

[1]M-GSkE: small-berry Muscat grape skin extract; [2]A-GSkE: Augustiatis grape skin extract; [3]TPC: Total phenolic content; [4]IC_{50}: Concentration for 50% scavenging of the ABTS radical cation or DPPH radical. Letter [a] in rows denote values of statistically significant difference. Results are expressed as mean ± SD in μM GAE.

3.3. Antiplatelet Activity and Plasma Oxidation Inhibition

Antiplatelet activity of M-GSkE was higher than A-GSkE (Table 3) as a lower concentration of μM GAE was required to inhibit PAF-induced platelet aggregation (19.7 ± 0.1 < 26.6 ± 0.2; $p < 0.05$). On the other hand, a concentration such as the μM GAE of A-GSkE was required to increase the lag phase time of the plasma oxidation curves by 50% was

lower than M-GSkE (1.1 ± 0.2 < 5.7 ± 0.3; $p < 0.05$), showing higher antioxidant activity of A-GSkE compared to M-GSkE ($p < 0.05$) toward copper-induced plasma oxidation (Table 3).

Table 3. Antiplatelet activity and plasma oxidation Inhibition.

Bioactivity	[1]M-GSkE	[2]A-GSkE
[3]$IA_{50\text{-}PAF}$ (μM GAE)	19.7 ± 0.1 [a]	26.6 ± 0.2
[4]$LTIC_{50\text{-}POX}$ (μM GAE)	5.7 ± 0.4 [a]	1.1 ± 0.2

[1]M-GSkE: small-berry Muscat grape skin extract; [2]A-GSkE: Augustiatis grape skin extract; [3]IC_{50}: concentration for 50% inhibition. [4]$LTIC_{50\text{-}POX}$: concentration for 50% lag time increase for plasma oxidation. Letter [a] in rows denote values of statistically significant difference. Results are expressed as mean ± SD in μM GAE.

3.4. Free Phenolic Profile by HPLC-DAD Analysis

The results from the HPLC-DAD phenolic analysis are presented in Table 4. Gallic acid, vanillic acid, ferulic acid, and ε-viniferin were detected in both extracts. The content of those four phenolics was higher in A-GSkE compared to M-GSkE ($p < 0.05$). Moreover, catechin, chlorogenic acid, and resveratrol were identified only in M-GSkE, while caffeic acid and Daidzein were identified only in A-GSkE.

Table 4. Free phenolic profile in methanolic extracts of grape skin samples.

Standard Phenolic Compounds	[1]M-GSkE Phenolic Compounds (μg/g) [3]	[2]A-GSkE Phenolic Compounds (μg/g)
Ferulic acid	13.2 ± 0.1 [a]	41.3 ± 0.1
Vanillic acid	12.2 ± 1.2 [a]	26.3 ± 1.7
Gallic acid	8.4 ± 2.9 [a]	16.6 ± 0.1
ε-viniferin	2.8 ± 0.3 [a]	3.6 ± 0.4
Catechin	59.8 ± 1.5	n.d.
Chlorogenic acid	43.8 ± 0.9	n.d.
Resveratrol	0.83 ± 0.13	n.d.
Caffeic acid	n.d.	19.8 ± 0.4
Daidzein	n.d.	16.8 ± 0.1
Tyrosol	n.d.	n.d.

[1]M-GSkE: small-berry Muscat grape skin extract; [2]A-GSkE: Augustiatis grape skin extract; [3]results are presented as the mean value of two independent analysis in μg of each phenolic per g of dried grape skin before extraction. Letter [a] in rows denote values of statistically significant difference.

Representative HPLC chromatographs are reported in Figure 1.

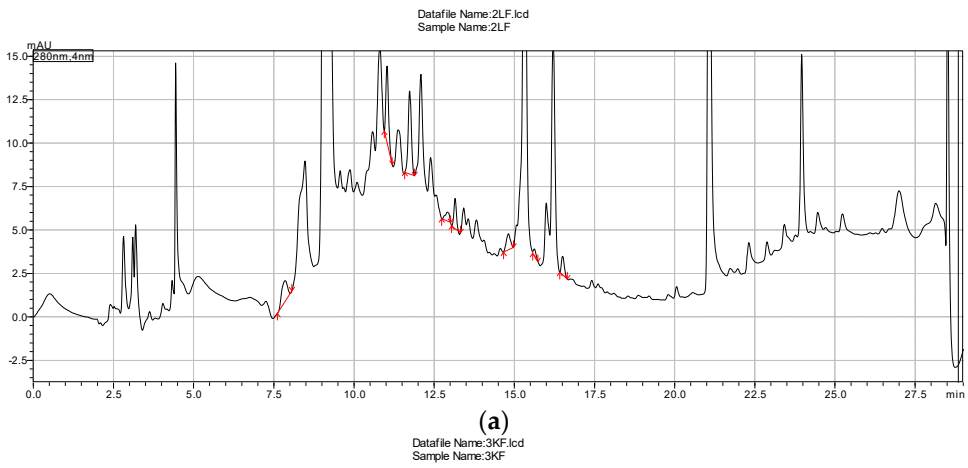
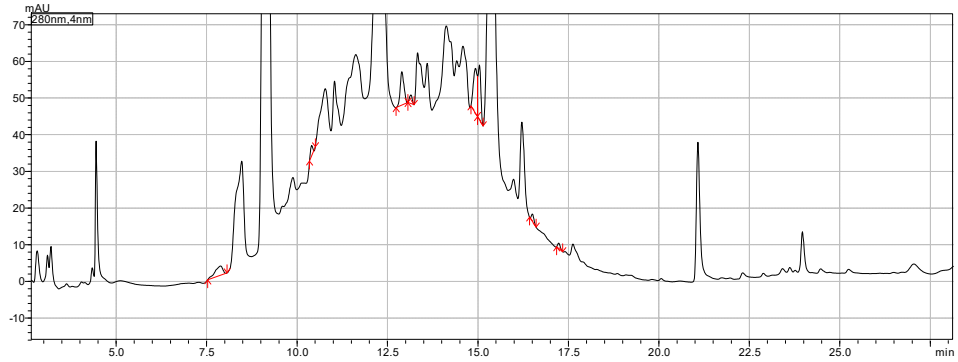


Figure 1. *Cont.*



(**b**)

Figure 1. Representative HPLC chromatographs at 280 nm of (**a**) M-GSkE and (**b**) A-GSkE along with chemical structures of identified compounds. Retention times in min were gallic acid, 7.8; catechin, 11.0; chlorogenic acid, 11.8; tyrosol, 12.2; caffeic acid, 12.9; vanillic acid, 13.1; resveratrol, 14.5; ferulic acid, 14.8; ε-viniferin, 16.4; daidzein, 17.3.

4. Discussion

In the context of the circular economy, researchers have tried to exploit wine by-products with the final goal of producing human health-promoting foods.

This fact is an opportunity for local communities that have unique varieties and species that can be used as raw materials. The exploitation of such raw materials can lead to unique food products or to the improvement of traditional foods of such regions. In this approach, the effort to exploit winemaking by-products, such as wine grape skins, in the North Aegean region offers the Greek islands of the North Aegean a tool for both economic development and public health prevention. This is consistent with recent research presenting that food value chains are widely realized as more fair alternatives to conventional supply networks [27–30].

Under this working hypothesis, the bioactivity of methanolic grape skin extracts of a white (small-berry Muscat) and a red (Augustiatis) winemaking grape species cultivated in Samos was investigated. We showed that methanol/water 60/40 (v/v) was a good choice in solvent mixture to produce extracts with higher antiradical activity based on the ABTS assay compared to other aqueous methanolic solvents (Table 1). Methanol is a solvent that has been used in various ratios with water to extract grape skin antioxidants [31]. Our results are in accordance with the work of Ćurko et al. that showed that the 62.7% of methanol in water acted as a solvent for optimized total phenolic extraction from grape skin pomaces through microwave-assisted extraction [32].

Both extracts exerted high total phenolic content. Augustiatis had 1.8-times higher phenolic content compared to small-berry Muscat ($p < 0.05$) (Table 2). More specifically, we found 2.12 ± 0.23 mM GAE in A-GSkE and 1.19 ± 0.13 mM GAE in M-GSkE. These values are equivalent to 36. 06 ± 3.9 and 20.24 ± 2.21 mg GAE/g, respectively. The results are consistent with other studies that have referred values of TPC ranging from 12.74 to 47.72 mg GAE/g [33–35]. Differences in the content of the total phenolics are attributed to the different grape varieties studied as well as to the differences in the methodologies used to produce the extracts.

Augustiatis also exerted 1.15- and 1.18-times higher antiradical activity based on ABTS and DPPH assays, respectively. The results showed $IC_{50\text{-ABTS}}$ values of 6.6 ± 0.3 and 7.7 ± 0.4 μM GAE for A-GSkE and M-GSkE, respectively. These values are equivalent to 213.54 ± 4.27 and 101.84 ± 8.56 μmol Trolox/g for A-GSkE and M-GSkE, respectively. The results are within the range 42.07 ± 5.93 to 447.27 ± 10.49 μmol Trolox/g of values reported in previous studies on grape skins of other varieties [33,36]. The $IC_{50\text{-DPPH}}$ values of 26.4 ± 1.0 and 31.2 ± 0.8 μM GAE for A-GSkE and M-GSkE, respectively, are equivalent to 224.37 ± 8.50 and 119.13 ± 3.05 μmol Trolox/g. $IC_{50\text{-DPPH}}$ values of our study are also

in agreement with previous studies on the grape skins of other varieties, which presented ranges from 79.71 ± 1.13 to 390.0 ± 4.3 μmol of Trolox/g [33,36].

Our results show that radical scavenging activity is corelated to the phenolic content ($p < 0.05$ for both ABTS and DPPH results) that is consistent with previous studies [31,37].

Both extracts exerted inhibitory bioactivity against in vitro copper induced plasma oxidation; however, Augustiatis was five-times more bioactive compared to small-berry Muscat ($p < 0.05$; Table 3). This result is in accordance with our results concerning the total phenolic content and antiradical activities in the two samples. According to our knowledge the inhibition of copper-induced plasma oxidation from grape skin samples has not been studied previously. Nonetheless, our results are supported by other studies in wine samples showing that the extent of LDL oxidation inhibition is directly related to the total phenolic content in the wine samples [38].

Concerning antiplatelet activity, small-berry Muscat exerted 1.34-times higher antiplatelet activity compared to Augustiatis ($p < 0.05$; Table 3). Antiplatelet activity against the thrombotic and inflammatory lipid mediator of PAF from the grape skin samples has not been previously examined, as far as we are aware. Nevertheless, our results are supported by studies in wine samples showing that the protective effect of a wine is independent of its color but is strongly associated with its microconstituent phenolic profile [39].

Indeed, the phenolics identified in the two extracts are different. Ferulic, vanillic, and gallic acid along with ε-viniferin were identified in both Augustiatis and small-berry Muscat. In those common phenolic compounds, Augustiatis presents higher contents ($p < 0.05$; Table 4). Catechin, chlorogenic, and resveratrol were identified only in small-berry Muscat, while caffeic acid and daidzein were identified only in Augustiatis.

Other researchers have also posited that the polyphenolic composition of grape skin extracts depend on the grape variety [40]. Although many phenolic compounds have been presented to have antiplatelet activities [41–45], our results show clearly that the type and concentration of phenolics existing in the extracts determine which of the samples will have higher antiplatelet activity.

5. Conclusions

There are several factors that must be considered before the commercial implementation of the recovery of value-added chemicals from food by-products can be considered. Improving the value of by-products from the wine industry will help cut down on expenses and recoverable materials. This is in line with European rules about the management of food by-products, which stress the need to reduce the quantity of by-products while simultaneously increasing their value.

Methanolic extracts of grape skin samples were tested for their total phenolic content (TPC) and their ability to scavenge free radicals, to prevent platelet aggregation, and to reduce plasma oxidation. The phenolic profile of methanolic grape skin extracts was noteworthy. Both extracts contained abundant phenolic components, including ferulic acid, vanillic acid, gallic acid, ε-viniferin, catechin, chlorogenic acid, resveratrol, caffeic acid, and daidzein.

This is the first time that small berry Muscut and Augustiatis grape varieties have been studied for their skin bioactivities. Methanol:water 60:40 (v/v) yielded extracts from the grape skins studied with higher antiradical activities based on the ABTS assay compared to methanol:water 70:30 or 80:20 (v/v). The total phenolic content and antiradical activities based on the ABTS and DPPH assays were higher for the skin sample extract of Augustiatis (A-GSkE) compared to that of small berry Muscut (M-GSkE). A-GSkE was also more active compared to M-GSkE toward the inhibition of copper-induced plasma oxidation. On the other hand, M-GSkE was more potent compared to A-GSkE toward platelet aggregation induced by the thrombotic and inflammatory lipid mediator of the platelet-activating factor (PAF). These bioactivities are determined by bioactive molecules in the obtained extracts, and the differences between A-GSkE and M-GSkE concerning the quantity and quality of bioactive phenolics may explain the obtained results. These findings demonstrate that

each variety excels in certain bioactivities. This information should be considered while planning the future valorization of food by-products such as grape skins. The present study highlights the nutraceutical potential of the grape skins of two unexplored winemaking grape varieties.

The considerable grape skin by-product presents an opportunity for the creation of novel functional foods or the refinement of current traditional products with superiority in consumer health protection. The results indicate that grape skins of small berry Muscut and Augustiatis could be the subject of a mixture design for the formulation of new enriched healthy animal or plant food products such as meat products, dairy products, bakery snacks, traditional pasta, spread products, beverages, or even wine with increased antioxidant and antiplatelet activities.

Increased consumer demand for such products would assist local economies in these locations by helping them establish a more solid economic basis for the future, and research in this area will continue to provide support to this endeavor.

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