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Article

Quality evaluation of winery by-products from Ionian Islands grape varieties in the concept of circular bioeconomy

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Abstract: The aim of this work was the study and evaluation of winery by-products in the framework of circular bioeconomy. Grape seeds and grape skins from Greek traditional Ionian Islands varieties were analyzed in an attempt to provide the appropriate basis for model development of their sustainable exploitation at a local or regional level. The wastes collected directly from the wineries immediately after the vinification process and analyzed by chromatographic and spectroscopic techniques. Also, annual production and yields were estimated. Grape seed oil quality was evaluated based on fatty acid methyl esters (FAMEs) composition. Grape skins phenolic fraction was extracted by an eco-friendly, non-toxic water-glycerol solvent system and were detected qualitatively. Also, total phenolic content (TPC) and antioxidant activity were measured. Based on estimated yields, our results demonstrate that winery by-products have the potential to promote the cyclical bioeconomy in a modern economic growth model that will reduce waste, and environmental costs as they can be reused as whole material in foods, dietary supplements, cosmetic ingredients, food colorants and preservatives.

Keywords: grape skins; grape seed oil; fatty acid methyl esters; total phenolic content; antioxidant activity; green extraction; circular bioeconomy

1. Introduction

Grape is one of the world's largest fruit crops, with more than 75 million tons is cultivated mainly as *Vitis vinifera* L. for wine production. Wine production it is important for the Greek economy, ranking the second most profitable industry of Greece after olives for the years 2015 and 2016 according to Food and Agriculture Organization (FAO) statistics [1]. Approximately, 35.9 million tons of by-products arise from wine, while the rest arise from grape juice production [2]. The year 2017, according to Ministry of Rural Development and Food, the Ionian Islands produced 1,179.25 tons of grapes for wine making and 175.12 tons of them were grape pomace [3]. All these wastes could acquire greater potential value if they utilized properly.

Wine industry produce a large volume of solid wastes, which mainly disposed in the environment causing economic and environmental problems [4, 5]. Winery wastes are produced continuously all year round and they could be hazardous for the environment [6]. Byproducts are typically characterized by high levels of chemical oxygen demand (COD) and biodegradability [7]. Specifically, grape pomace consists of grape seeds and skins and they remain after pressing and the fermentation of vinification processes. Grape pomace has high organic matter and they could cause pollution to the soils and water.

Grape seeds are a valuable source of oily constituents like sterols, triglycerides and fatty acids. Grape seed oils have high abundance to poly- and monounsaturated fatty acids (PUFAs and MUFAs), with PUFAs to comprise the majority of fatty acids. The content

of oil can yield from 5.85 to 22.4% (w/w), and this yield can be related with the cultivar, variety, differ from year to year and extraction method [8- 10]. Grape skins are characterized by high-phenolic contents. The phenolic composition depends on the variety of grapes and vinification conditions. Anthocyanins, catechins, flavonol glycosides, phenolic acids and stilbenes are the main phenolic compounds [11]. These compounds are responsible for some of the most important wine properties and simultaneously have antioxidant activities [12]. Many studies have evaluated the methods for the valorization of winery wastes. These studies have been focused on antioxidant and health-promoting activities [4]. Phenolic compounds react to the free radicals and neutralizing them with beneficial effect in anti-inflammatory, cardioprotective and anticarcinogenic [13]. The extraction of phenolic compounds from by-products can be done with many organic solvents such as methanol, ethanol, acetone and ethyl acetate [12].

The islands, like the Ionian Islands, are sensitive systems in relation to the mainland. This is due to many factors such as their small size, their peculiar environment and their isolation in relation to the mainland. The small size, in terms of area and population, implies a limited variety and quantity of natural resources, reducing the possibilities for large-scale productive activities. Also, their distance from the urban centers in combination with the traffic difficulties, due to the sea, significantly affects the degree of isolation. The result of these characteristics is the creation of fragile ecosystems with uncontrolled environmental influences and limited development potential. The circular economy could contribute positively to the solution of the insularity problem. The transition to a cyclical bioeconomy presupposes the reuse, repair, renewal and recycling of materials and products. What was considered "waste" can be turned into raw material. Strengthening cooperation across the supply chain can reduce both costs and waste and the environmental burden. Developments in environmental innovation ensure new products, processes, technologies and organizational structure.

Valorization of waste biomass for the recovery of phytochemicals should include processes that generate far less or even zero further waste. Otherwise no concept of “green” or “sustainable” could be substantiated. Thus, research should focus on the discovery and design of extraction processes, which will allow the use of alternative solvents and renewable natural products, and ensure a safe and high quality extract/ product [14]. Glycerol, is a bio-liquid considered a by-product of the biodiesel industry, and simultaneously has not been used widely for extraction purposes. In addition, it constitutes a green and well-established sustainable solvent. [15, 16]. Utilization of grape pomace as a whole product or combination with green solvents to extract bioactive compounds can be used in the food and cosmetic industry and add value in final products [17-19].

The goal of this study was the management of winery by-products providing an efficient solution to their reduction promoting the circular bioeconomy. For this purpose, was carried out a quality study of the grape pomace. Extracts were obtained by non-toxic environmental friendly solvents and the fractions were evaluated for their antioxidant activity. For this purpose, were selected traditional grape varieties of Ionian Islands which give wines of protected designation of origin (PDO) and have not been adequately are studied. Finally, must be highlighted that analyses were based on waste just as they were taken from the wineries to provide a realistic view and the promotion of cyclical bioeconomy. This research will also serve as a valuable contribution to a deeper investigation of the understudied of sustainable management of agro waste in Ionian Islands.

2. Materials and Methods

2.1. Chemicals

All the solvents used were extra purity (>99.5%) including water, glycerol and n-hexane, cyclohexane and methanol. Phenolic standards with purity of 98–99% (cinnamic acid, gallic acid, caftaric acid, catechin and epicatechin, epicatechin gallete, rutin, quercetin, kaempferol-3-glucoside, p-coumaric acid and isoharmentin-3-glucoside) were purchased

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from Aldrich (Steinheim, Germany) used for identification in MS. Folin-Ciocalteu reagent used for TPC measurement, as well as caffeic acid. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis- (3- ethylbenzothiazoline-6-sulphonic acid) (ABTS) used for the free radicals preparation tests, as well as Trolox.

2.2. Plant Material

Winery by-products were provided by Gentillini winery and Vineyards, the Robola Cooperative of Cephalonia, Ktima Grampsa, Ktima Theotoki and Robotis wineries. Thirty-six samples were analyzed and some of them came from the PDO Robola in Cephalonia. The rest of them were varieties from Pavlos, Avgoustiatis, Robola, Goustolidi, Savvatiano, Cabernet Sauvignon, Kakotrygis, Sauvignon Blanc, Tsaousi, Mavrodaphne, Vardea, Vertzami.

2.3. Moisture Removal

Initial moisture of crude grape pomace was estimated and expressed in % (w/w). The average moisture of estimated at 73%. Drying process of samples was then carried out in dark on large non-absorbent surfaces at 35 °C assisted by air flow until a final moist content of 13% was achieved. Duration of drying process was two days in average and followed by separation of grape seeds from the skins using a series of sieves. Ratio skins to seeds for every sample was also specified. Seeds and skins of each sample were sealed in polypropylene bags and stored at -20 °C until their usage.

2.4. Oil Extraction from Grape Seeds

Grape seeds were powdered with mixer (Philips HR 2074, N.V.) for 20 s. The crushed grape seed powder was continuously extracted with n-hexane in a Soxhlet apparatus at 70 °C for 6 h. The analogy of crushed seeds to n-hexane was 1 to 10 (w/ v). Then, n-hexane fraction was evaporated to dryness under reduced pressure at 35 °C and residuals removed under nitrogen flow.

2.5. Phenolic Extraction from Grape Skins

Grape skins were powdered with mixer (Philips HR 2074, N.V.) for 2 min. During grinding were 15 s rest periods to avoid overheating of the samples. The crushed grape skins powder was continuously defatting with n-hexane with magnetic stirrer at 600 rpm for 30 min at room temperature (25 °C). The analogy of skins powder to n-hexane was 1 to 10 (w/ v) and the process was repeated three times [12]. Then, defatted samples extracted with water and glycerol (80:20 v/ v) at 600 rpm for 60 min at room temperature (25 °C). The ratio of skins powder water and glycerol (80:20 v/ v) followed was 1 to 20 (w/ v) and the process was repeated three times. The three fractions were filtered through a 0.45 μm filter and stored at -20 °C until further analysis.

2.6. Determination of Total Phenolic Content

TPC was estimated using the Folin- Ciocalteu reagent [20]. Absorptions were measured at 765 nm. The TPC concentration (C_{TPC}) was calculated and expressed as mg caffeic acid equivalents per mL extract (mg CAE mL^{-1}). TPC yield (Y_{TPC}) was calculated as mg caffeic acid equivalents per g of dry weight (mg CAE g^{-1}), using the following equation:

$$Y_{TPC}\left(\frac{mg\ \text{caffeic acid}}{g\ \text{dry weight}}\right) = C_{TPC} \times \frac{V}{m}$$

Where (V) is the volume of the extraction and (m) the dry weight of plant material (g).

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2.7. Antioxidant Activity of Grape Seed Oils and Grape Skins Extracts

Antioxidant activity was estimated using DPPH and ABTS assay and A_{AR} was also calculated [21-24]. Absorptions were measured in triplicate at 517 nm and 734 nm for DPPH and ABTS respectively and expressed as mg trolox equivalents g^{-1} dry weight. The percentage of inhibition was calculated according to the following formula:

$$DPPH\ (Inhibition\ \%) = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100$$

$$A_{AR}\ (\mu mol\ DPPH/\ g\ dw) = \frac{C_{DPPH}}{C_{TPC}} \times \left(1 - \frac{A_{515\,(f)}}{A_{515\,(i)}}\right) \times\ Y_{TPC}$$

Where C_{DPPH} is the initial molar concentration of DPPH (μmol L^{-1}), $A_{515(f)}$: sample's absorbance and $A_{515(i)}$: absorbance of blank sample.

2.8. Analysis of FAMEs in Grape Seed Oils by GC-MS

The analysis of FAMEs was performed using a Trace Ultra gas chromatograph (GC) (Thermo Scientific), coupled to a mass spectrometer (MS) (DSQII, Thermo Scientific). The column used was a TR-5MS (30 m × 0.25 mm i.d., 0.25 μm film thickness) and the carrier gas was helium, at 1 mL min^{-1} rate. The oven temperature was adapted to 110 °C and then was increased at 205 °C with a rate of 4 °C min^{-1}, followed by an increment of 1 °C min^{-1} up to 215 °C and, up to 250 °C with a step of 4 °C min^{-1}. Finally, the temperature of 250 °C was kept constant for 15 min. The transfer line and injector temperatures were maintained at 260 and 220 °C, respectively. The injection volume was 1 μL, in a split-less mode. The peak identification carried out with the Wiley 275 mass spectra library, its masses spectral data and arithmetic index provided by Adams 0.7 HP.

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

K_{232}, K_{268}, and K_{270} extinction coefficients were measured from the absorption of the samples in the UV region at 232, 268 and 270 nm respectively, with a UV-Vis (Cary 60, Agilent spectrophotometer). The samples were prepared according to the ISO 3656:2011.

2.10. HPLC-DAD and LC-MS Analysis

Phenolic extracts were analyzed on a high pressure liquid chromatography (HPLC) Agilent 1100 series (Agilent Corporation, MA, USA) with diode array detector (DAD). The system was connected to a computer and HP Chemstation software.

Also, analyzed on a Shimadzu LC/MS-2010A equipped with an LC-10ADvp binary pump, a DGU-14A degasser, a SIL-10ADvp autosampler, an SPD-M10Avp Photo Diode Array Detector and a quadrupole mass detector (MSD) with an electron spray ion source (MS-ESI, Electrospray Ionization). The system was connected to a computer and Shimatzu version 3.40.307 software for the chromatographic processing. The detector was set to negative ion operation mode under these conditions: Ionization source temperature CDL (curved dissolvation line) 300 °C, mist gas flow (N_2) 1.5 L min^{-1}, drying gas pressure (N_2) 0.1 MPa (10 L min^{-1} flow), heat block temperature 300 °C, mist area potential -2.5 kV, CDL voltage - 20V, detector voltage - 1.55 kV, scan area: 50-1000 m/z and scan speed 6000 amu s^{-1}.

A reversed-phase column Supelco (Discovery HS C18), length 250 mm, internal diameter 4 mm with material porosity of 5 μm was used and it eluted the analytes at a flow rate of 1 mL min^{-1}. The following gradient of mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in methanol) was used for the analysis: The program followed was from 0-1 min, 5% solvent (B), 1-5 min, 10% solvent (B), 6-15 min, 33% solvent (B) 16-25 min, 41% solvent (B), 26-35 min, 62% solvent (B), 36-42 min, 66%

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solvent (B), 43-55 min, 100% solvent (B), 56-65 min, 5% solvent (B). Chromatograms were recorded at wavelengths 280, 320, 360 and 520 nm [21].

2.11. FTIR Spectroscopy

Fourier-transform infrared (FTIR) spectra were obtained using a Thermo Nicolet 6700 FTIR, (Thermo Electron Corporation, Madison, WI, USA) equipped with a deuterated triglycine sulfate (DTGS) detector. The spectra were obtained in diffuse reflectance infrared fourier transform spectroscopy (DRIFTS) technique. The speed of the interferometer moving mirror was 0.6329 mm s^{-1}. Spectra were recorded with a resolution of 4 cm^{-1} and 100 scans. Before the analysis of each sample, the background was recorded. Triple FTIR spectra of each sample were obtained, using a different sub-sample each time.

FTIR spectra were smoothed using Savitsky–Golay algorithm and their baselines were corrected. These pre-treatments were performed with "automatic smoothing" (5-point moving second-degree polynomial) and "baseline correction" (second-degree polynomial, twenty iterations) functions. Finally, using the "statistical spectra" function, the mean of three spectra for each sample was calculated it was normalized (absorbance maximum value of 1). Spectrum processing was performed using the software (OMNIC ver.9.1, Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.12. Raman Spectroscopy

A DeltaNu Advantage 785 visible–infrared Raman spectrometer (DeltaNu Inc., Laramie, WY, USA) equipped with a 785 nm diode laser for excitation with a maximum output power of 71.6 mW was used to record the spectra. Each spectrum was a 10 s acquisition over the spectral range of 2000–200 cm^{-1} using a resolution of 8 cm^{-1}. The spectrometer was accompanied by NuSpec software. Raman spectra processing was performed as FTIR spectra.

2.13. Statistical Analysis

All the experiments were done in triplicates and the results were given as mean ± standard deviation (SD).

3. Results

3.1. Grape Seed Oils Analysis

The grape seed oils yields differ for each grape variety tested. Higher yields were assigned to Robola from Zakynthos, yielding 8.77± 0.18% w/ w, followed by Cabernet Sauvignon from Corfu, 8.11± 0.18 w/ w. Lower yields were found in cultivars of Robola coming from the PDO of Robola in Cephalonia, ranging from 5.26± 0.33 to 7.01± 0.85% w/ w. Other authors have pointed out that yields are close related to two factors, including the variety tested [19] and the extraction method followed [9].

Grape seed oils had a valuable source of unsaturated fatty acid (SFAs) profile. Particularly, the FAMEs composition of the grape seed oils varied between the different varieties and cultivars. Linoleic fatty acid (C18:2) was the most abundant, followed by oleic fatty acid (C18:1), palmitic fatty acid (C16:0) and last, stearic fatty acid (C18:0). Other fatty acids found in the samples in smaller amounts were myristic (C14:0) and palmitoleic (C16:1). Linoleic fatty acid ranged from 53.28± 1.24 to 57.05± 1.40% in Robola cultivars coming from the PDO of Robola in Cephalonia (**Figure 1**). Other varieties appeared to be higher in abundance of linoleic fatty acid. For example, Tsaousi and Sauvignon Blanc from Cephalonia, Cabernet and Sauvignon Blanc from Corfu and Robola from Zakynthos had 60.95±1.65%, 60.82± 2.45%, 59.66± 0.84%, and 59.26± 0.91%, respectively. On the other hand, oleic fatty acids were the second higher in abundance constituent. For example, cultivar of Robola from Cephalonia had content from 22.41± 0.76 to 25.44± 1.36%. High

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oleic fatty acid content combined with low linoleic fatty acid content appears to be a feature common for grape seed oils, as other studies have mentioned [19].

For the rest of the varieties were tested oleic fatty acid ranged from 17.55± 2.37% in Sauvignon Blanc from Cephalonia to 21.74± 0.28% in Goustolidi from Zakynthos. Both of linoleic and oleic fatty acids totaling 78- 82% of FAMEs, that reminds the fatty acid composition of safflower oil, that is related to the genotype and the environment chosen [26, 27].

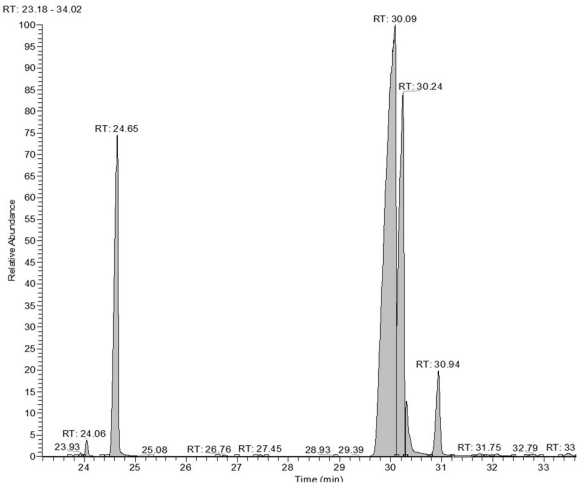

Figure 1. GC of Robola grape seed oil. Linoleic fatty acid (C18:2) is the major peak at retention time (Rt) 30.09, followed by oleic fatty acid (C18:1) at Rt 30.24, palmitic fatty acid (C16:0) at Rt 24.65 and stearic fatty acid (C18:0) at Rt 30.94.

3.2. Grape Seed Oils Antioxidant Activity

Antioxidant activity of grape seed oils were estimated by DPPH and ABTS assays. The varieties of Tsaousi from Cephalonia, Goustolidi from Zakynthos and four of the cultivars of the PDO of Robola in Cephalonia exhibited more than 60% scavenging effect of the DPPH radicals, while the before mentioned samples inhibited more than 90% of the effect of the $ABTS^{\bullet+}$ radical, except in the case of Tsaousi from Cephalonia, where the scavenging effect of the $ABTS^{\bullet+}$ radical was 64.8%. The rest of the grape seed oils tested for their antiradical activity showed scavenging effect of the DPPH radicals ranging from 42.95 to 56.25%, while they succeed better in eradicating the $ABTS^{\bullet+}$, with an inhibition rate from 88.16 to 94.20%.

Similarly, to previous research [28, 29], results of this study indicate that grape seed oils are poor in phenolics due to the low solubility of phenolics in the lipid fraction, as most of the phenolic compounds remains in the defatted grape seed particles whose concentration in phenolics is at least 100-fold higher than the phenolic concentration in the oil [30]. TPC in grape seed oils by soxhlet extraction found to vary from 0.024 – 0.053 mg CAE g^{-1} [31, 32].

From the results, we conclude that the antioxidant activity of the extracts is influenced by the assay, the extraction method and the chemical compounds they contain. Other probes have greater sensitivity to aqueous extracts such as DPPH assay, while others have greater sensitivity to lipophilic extracts such as ABTS. Finally, the determination of the antioxidant activity of a separate component of the extract is almost impossible due to the complexity of its composition and the synergistic action between the components. For this reason, the determination of antioxidant activity with at least two methods of choice is a mandatory step in order to have comparable results and link the content of phenolic components that have antioxidant properties to antioxidant activity.

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3.3. Grape Seed Oils Spectroscopic Indices

Spectroscopic indicator, K_{232} was more than 2.50 for three samples, including two of the Robola cultivars of the PDO of Robola in Cephalonia and Tsaousi from Cephalonia. In all the other samples K_{232} were less or equal to 2.50. Moreover, six of the grape seed oils ΔK were equal to 0.1 and for the other six more than 0.2. According to the EU regulation [33] oils with ΔK above 0.1 are not subjected in the category "extra virgin/ virgin olive oil". K indices can be misleading if used as the only criterion of the oil quality other than olive oil and therefore must be combined with the other quality parameters as well.

3.3. Raman Spectroscopy in Grape Seed Oils

A representative Raman spectrum from grape seed oil is presented in **Figure 2**. The grape seed oils gave two strong peaks, one in 1655 cm^{-1} and the other in 1444 cm^{-1}. Peak at 1655 cm^{-1} mentioned to unsaturated cis double bonds, while the peak at 1444 cm^{-1} belonging to (-CH_2) scissor and twist vibration of fatty acids. That's peaks tend to be stronger as the chain length of the fatty acids increases [34]. The peaks between 1400 and 800 cm^{-1} belonging to aliphatic stretches [35].

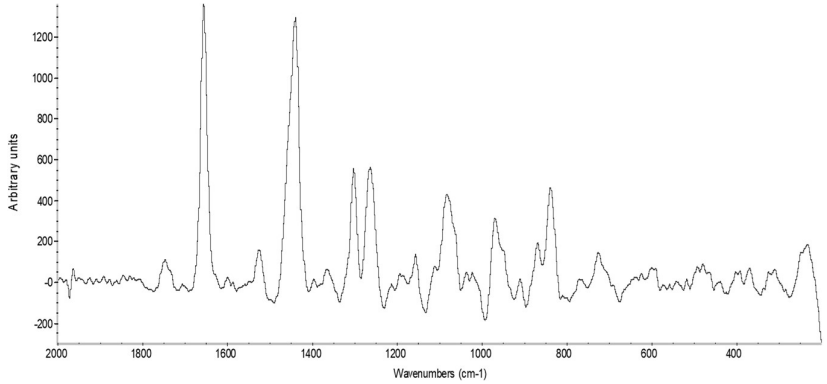

Figure 2. Raman spectrum of Robola grape seed oil from Cephalonia.

3.4. Grape Skin Analysis

Pretreatment of the samples before extraction had an important role in the recovery of bioactive compounds for further analysis. Qualitative determination of phenols according to their structure showed a characteristic absorption spectrum in UV-Vis. In particular, hydroxybenzoic acids, flavonols and procyanidins were detected at 280 nm, stilbenes, hydroxycinnamic acids and their esters at 320 nm, flavonols and their glycosides at 360 nm. UV-Vis spectra of flavonoids shown the two absorption bands I and II. Zone I had absorbance range 300-370 nm due to the structure of rings B and C while band II had absorbance range 250-300 nm and due to A-ring of flavonoids. In absorbance range 260-280 nm we also confirmed the existence of phenolic compounds and particular phenolic acids [25]. Finally, absorptions at 520 nm, were attributed to anthocyanins.

Determination of phenolic compounds were identified by LC-MS analysis, comparing mass spectrum and the UV spectrum with standards. Peaks were attributed to monomeric 3-flavanols as well as monomeric, dimeric and trimeric proanthocyanidins. Specifically, Robola grape skins extracts were rich in proanthocyanidins, catechin, epicatechin, glycoside of kaempferol, and 3-glucuronide of quercetin (**Table 1**).

Grape skins from white varieties have higher content of trans-caftaric acid, against to red grapes [36]. Quercetin-3-O-glucuronide and quercetin-3-O-glucoside, remain at similar high levels in all varieties [37]. The content of myricetin has been not detected in most of white varieties. This could be due to the absence of the enzyme flavonoid-3',5'-hydroxylase in white grape varieties [38, 39]. Tannins represent a significant content of the bioactive phytochemicals in vinification residues and available records describe the

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presence of procyanidin dimers B1, B2, B3, and B4 and procyanidin trimers C1, C2, and C3. Grape skins extracts contain high oligomeric proanthocyanidins [40], which can combine with gallic acid to form gallate esters, and ultimately glycosides [41].

The analysis of winery by-products suggests differences in phenolic content from red and white varieties. This observation enables us to manage the red and white varieties as separate materials.

Table 1. Tentative identity of major polyphenols of the Robola variety extract from Cephalonia.

Peak Rt	UV-Vis	$[M-H]^-$	Tentative Identity
7.4	274	146	cinnamic acid
9.5	215; 270	168	gallic acid
13.5	217	330	monogaloglucose
14.8	218; 278	576	procyanidin dimer
15.0	217; 277	576	procyanidin dimer
15.3	278	577	procyanidin dimer
15.5	286; 328	310	caftaric acid
15.9	218; 277	865	procyanidin trimer
16.4	220; 279	576	procyanidin dimer
16.8	220; 277	576	procyanidin dimer
17.3	278	288	catechin
17.8	278; 375	576	procyanidin dimer
18.3	223; 271	443	epicatechin gallate
19.1	219; 278	865	procyanidin trimer
19.2	222; 278	896	catechin trimer
19.3	222; 278	577	procyanidin dimer
20.5	278	289	epicatechin
25.0	226	163	p-coumaric acid
30.5	225; 354	476	quercetin-3-glucuronide
31.1	226; 355	609	rutin
33.5	264; 348	446	kaempferol-7-O-glucoside
34.2	265; 349	446	kaempferol-3-O-galactoside
34.4	226; 350	477	isorhamnentin-3-O-glycoside
37.7	373	301	quercetin

3.5. Grape Skin Antioxidant ctivity

The results of antioxidant activity displayed in **Table 2** and confirmed the high TPC performance of grape skins.

Table 2. TPC and antioxidant activity of grape skins extracts.

Variety	Region	Total Phenolics		Antioxidant Activity			
		[a]C_{TPC}	[b]Y_{TPC}	[c]DPPH	[d]DPPH	[e]ABTS	[f]ABTS
Sauvignon Blanc	Cephalonia	0.12 ± 0.01	7.70 ± 0.87	0.60	29.55	24.49 ± 1.18	42.16
Tsaousi	Cephalonia	0.18 ± 0.01	11.07 ± 1.52	0.78	33.11	32.30 ± 1.43	55.68
Robola	Cephalonia	0.20 ± 0.01	12.15 ± 1.16	0.70	21.42	55.52 ± 0.85	95.88
Robola	Zakynthos	0.28 ± 0.01	17.27 ± 0.96	1.14	40.72	60.44 ± 1.29	104.39
Goustolidi	Zakynthos	0.47 ± 0.01	28.36 ± 0.65	0.90	42.73	77.28 ± 0.86	133.55
Robola	Cephalonia	0.21 ± 0.01	12.86 ± 1.00	0.65	19.94	63.86 ± 0.28	110.33
Robola	Cephalonia	0.35 ± 0.01	21.39 ± 0.87	1.05	31.16	98.04 ± 0.14	169.49
Robola	Cephalonia	0.27 ± 0.01	16.35 ± 0.73	0.86	25.93	86.94 ± 0.24	150.28
Robola	Cephalonia	0.21 ± 0.01	12.69 ± 0.72	0.53	16.75	58.74 ± 1.05	101.45
Mavrodaphne	Cephalonia	0.45 ± 0.01	27.14 ± 1.14	1.20	35.20	97.06 ± 1.33	167.79

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Robola	Cephalonia	0.18 ± 0.02	11.14 ± 1.21	0.46	14.87	43.35 ± 3.85	74.82
Robola	Cephalonia	0.29 ± 0.02	17.37 ± 1.33	0.79	23.89	71.37 ± 2.20	123.32
Avgoustiatis	Zakynthos	0.34 ± 0.01	20.43 ± 0.78	0.88	26.39	70.67 ± 0.70	122.11
Paul	Zakynthos	0.36 ± 0.01	21.92 ± 0.85	1.04	30.72	85.31 ± 0.24	147.46
Avgoustiatis, Skiadopoulos	Zakynthos	0.37 ± 0.02	22.61 ± 1.22	1.43	36.57	90.07 ± 2.15	155.69
Avgoustiatis, Katsali	Zakynthos	0.33 ± 0.02	20.20 ± 1.27	1.15	33.99	86.80 ± 0.90	150.04
Kakotrygis	Corfu	0.32 ± 0.01	19.48 ± 0.85	1.14	33.70	75.52 ± 2.45	130.51
Cabernet Sauvignon	Corfu	0.56 ± 0.01	33.70 ± 0.65	1.47	42.73	91.09 ± 0.62	157.46
Syrah	Corfu	0.16 ± 0.01	10.00 ± 0.87	0.40	15.89	46.37 ± 0.76	80.05
Matzavi	Corfu	0.19 ± 0.02	11.51 ± 1.52	0.53	18.87	54.35 ± 2.08	93.86
Robola	Corfu	0.34 ± 0.01	20.78 ± 0.96	0.88	27.69	86.29 ± 0.83	149.15
Moschato white	Corfu	0.19 ± 0.01	11.68 ± 0.65	0.44	19.43	67.61 ± 1.98	116.81
Mavrodaphne	Cephalonia	0.42 ± 0.01	19.40 ± 0.62	1.43	22.17	62.37 ± 1.01	107.75
Avgoustiatis, Pyrarnis	Zakynthos	0.43 ± 0.01	25.98 ± 0.77	1.22	38.10	98.62 ± 0.04	170.50
Vertzami	Lefkada	0.33 ± 0.01	20.37 ± 0.93	1.79	29.79	90.60 ± 0.46	156.61
Vardea	Lefkada	0.23 ± 0.01	13.87 ± 0.93	0.48	19.23	71.34 ± 4.36	123.27
Pavlos, Cardinal, Zambella	Zakynthos	0.24 ± 0.01	14.49 ± 0.63	0.57	24.83	65.29 ± 1.52	112.80

[a]C_{TPC} (mg mL^{-1}) ± SD: Concentration of TPC expressed as mg CAE mL^{-1} of extract
[b]Y_{TPC} (mg g^{-1}) ± SD: Yield TPC expressed as mg CAE g^{-1} dry weight.
[c]DPPH (A_{AR}): A_{AR} expressed as μmol of DPPH g^{-1} of dry weight.
[d]DPPH (mg trolox g^{-1}): The inhibition of free radical DPPH expressed as mg trolox equivalents g^{-1} dry weight.
[e]ABTS (I % ± SD): The % inhibition of free radical ABTS
[f]ABTS (mg trolox g^{-1}): The inhibition of free radical ABTS expressed as mg trolox equivalents g^{-1} dry weight.

3.6. FTIR and Raman Spectroscopy of Grape Skins

A representative FTIR spectra from grape skin sample is presented in **Figure 3**. The assignments of the major peaks are shown in **Table 3**. It was observed that the spectra showed significant similarities. The samples consist of water, protein, fat, organic acid, sugar, nitrogen compounds and flavonoids.

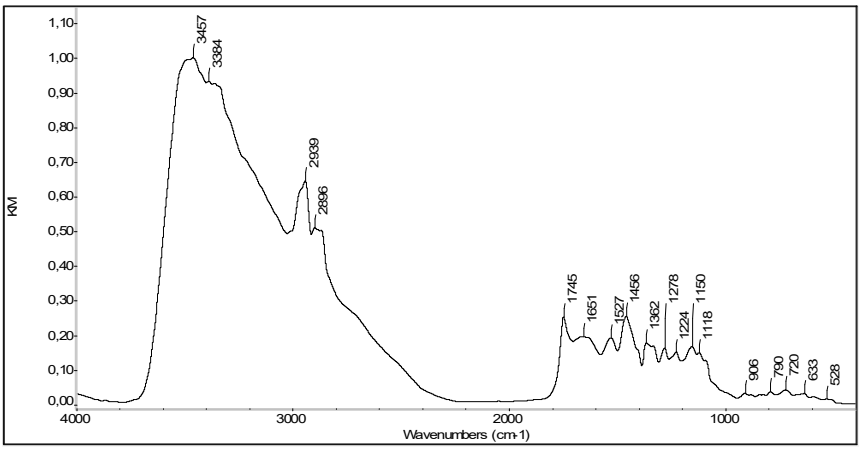

Figure 3. Mean FT-IR spectrum derived from Robola sample.

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Table 3. Main peaks of the FT-IR spectrum derived from Robola sample.

Wavelengths (cm^{-1})	Functional Group	Peak performance	Assignment	Reference
~ 3457	O-H	Sugars	Stretching	[42-44]
~ 3384	C-N	Proteins	Stretching	[42]
~ 2939	C-H ($-CH_2$)	Lipids	Symmetrical Stretching	[42, 43]
~ 2896	C-H ($-CH_2$)	Lipids	Asymmetric Stretching	[42, 43]
~ 1745	C=O; -COOR	Pectins; Triglyceride ester linkages; Amide I	Stretching	[42], [45]
~ 1651	C=O; $-COO^-$	Triglyceride ester linkages	Asymmetric Stretching	[45]
~ 1527	C-N; N-H	Proteins; Amide II	Stretching, Bending	[42]
~ 1456	C-N; $-CH_2$	Amide III, Lipids	Stretching, Bending	[42]
~ 1362	$-CH_3$	Lipids	Symmetrical bending	[42]
~ 1278	C-O-C	Lipids	Asymmetric Stretching	[42]
~ 1150	C-O; C-O-C	Polysaccharides; coutin	Stretching	[44]
~ 1118	C-O-C	Sugars; polysaccharides	Stretching	[43]
~ 790	C-C	Lipids	Stretching	[44]
~ 720	$-CH_2-$	Sugars	Swing	[44]
~ 633	C-H	Aromatic ring	Bending	[46, 47]

Respectively, Raman spectra from grape skin extract is presented in **Figure 4**. The assignments of the major peaks are shown in **Table 4**. It was observed that the spectra showed significant similarities. The samples consisted of phenolic compounds distinguished in non-flavonoid phenols and in flavonoid phenols. In particular, the extracts contained phenolic acids, flavonols, flavanones, tannins, and anthocyanins.

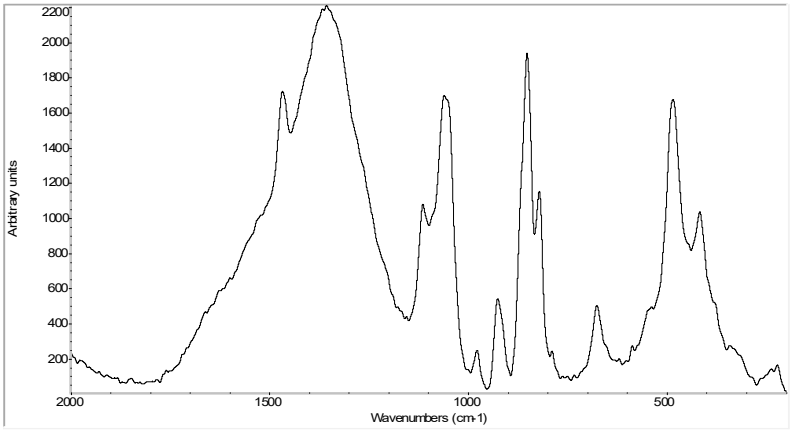

Figure 4. Mean Raman spectrum derived from Robola sample.

Table 4. Main peaks of the Raman spectrum derived from Robola sample.

Wavelengths (cm^{-1})	Functional Group	Peak performance	Assignment	Reference
~ 673	C=O	Monosubstituted benzene	Deformation	[46, 47]
~ 786	C-C; $-CH_2$	n - substituted benzene	Bending	[47]
~ 819	$-CH_2$	n - substituted benzene	Bending	[47]
~ 850	C-C	Alkane	Bending	[46]
~ 924	C-CH_3	Alkanes off-plane bending	Bending	[48, 49]
~ 975	C-CH_3	Alkane	Bending	[49, 50]
~ 1058	Benzene	Disubstituted benzene derivatives	Bending	[48, 49]
~ 1112	C-C; C-O	Sugar	Bending	[48]

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~ 1364	C-H; -CH_3; -OH,	Alkanes, Phenols	Stretching	[43]
~ 1466	C-H; -CH_3; C=C	Alkanes, Phenols	Stretching	[43]
~ 1628	C=C	Alkene, Aromatic ring	Bending	[43]
~ 1849	C=O	5-membered cyclic anhydrides	Bending	[43]

4. Discussion

Chemical analysis of grape pomace showed that they consist by valuable phytochemicals like PUFAs, MUFAs and polyphenols. These fatty acids can balance the PUFAs/ saturated fatty acids (SFAs) ratios of the human diet [51]. Furthermore, studies have shown that a diet enriched in polyphenols has multiple benefits for human health such as cardiovascular and coronary heart diseases [52, 53], diabetes [54] and anti-inflammatory activity [55, 56].

In past research was tried to develop foods, enriched by grape pomace either as extracts or as whole powder. Cereal and dairy products will be able to have an easier use for enrichment [57- 59]. Grape pomace was satisfactorily used in cheese manufacturing [60, 61], marmalade or candies [62], salad dressing [63], and tomato puree [64]. Meat products are the food categories in which these byproducts have been most widely used to prevent lipid oxidation. They have been applied in beef [65], pork [66, 67], chicken [68], turkey [69], goat [70] and buffalo [71]. Also, grape seed oil was proposed as an innovative food ingredient in various foods formulations improving their nutritional properties [72]. The incorporation of grape seed oil (up to 10%) was proposed to improve the fatty acid profile of frankfurters [73]. Otherwise, it can be used to cosmetics as it has moisturizing properties [74].

The availability of winery by-products in Ionian Islands according to statistical data of Greek Ministry of Agriculture is estimated in total at 1,038.87 tons per year. Cephalonia Island contributes with more than a half (53%) (**Figure 5)**. Moreover, more details of mass balance are presented in **Table 5**.

Table 5. Mass balance of winery by-products from Ionian Islands.

Ionian Islands	Variety	Annual production (tn)	Grape pomace (tn)[a]	Grape seeds (tn)[a]	Grape skins (tn)[a]	Grape seeds oil Yeild (L)
Zakynthos	**Pavlos**	10.910	1.091	0,592	0.499	47.498
	Avgoustiatis	72.660	6.771	4.441	2.331	356.224
	Robola	5.030	0.585	0.339	0.246	27.209
	Goustolidi	10.865	1.337	0.426	0.912	34.141
	Savvatiano	5.750	0.528	0.187	0.179	14.968
Corfu	**Robola**	22.220	2.136	0.624	0.760	50.045
	Cabernet Sauvignon	4.556	0.370	0.218	0.152	17.460
	Kakotrygis	17.794	1.191	0.733	0.458	58.833
Cephalonia	**Sauvignon Blanc**	0.800	0.119	0.042	0.077	3.398
	Tsaousi	57.379	6.539	4.518	2.022	362.384
	Robola	393.573	45.501	33.846	11.656	2715.025
	Goustolidi	38.245	3.392	2.218	1.174	177.925
	Mavrodaphne	61.415	5.747	2.533	1.992	203.179
Lefkada	**Vardea**	71.654	6.445	5.605	0.840	449.603
	Vertzami	266.014	27.702	17.168	10.534	1377.140

[a]Dry mass balance

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Based on estimated yields, a production of 5,895.03 L per year of grape seed oil will providing higher additive value. The minimized dry mass provides the advantages of easier and more cost efficient transportation both inside the islands and especially to the mainland. In addition, their transport time will be much shorter without particularly high costs. The amount of 107.32 tons of grape seeds and skins that can been reused as whole material in food of pharmaceutical companies contributing directly to the circular bioeconomy. These results underline also the importance for a management plan of waste in Ionian Islands.

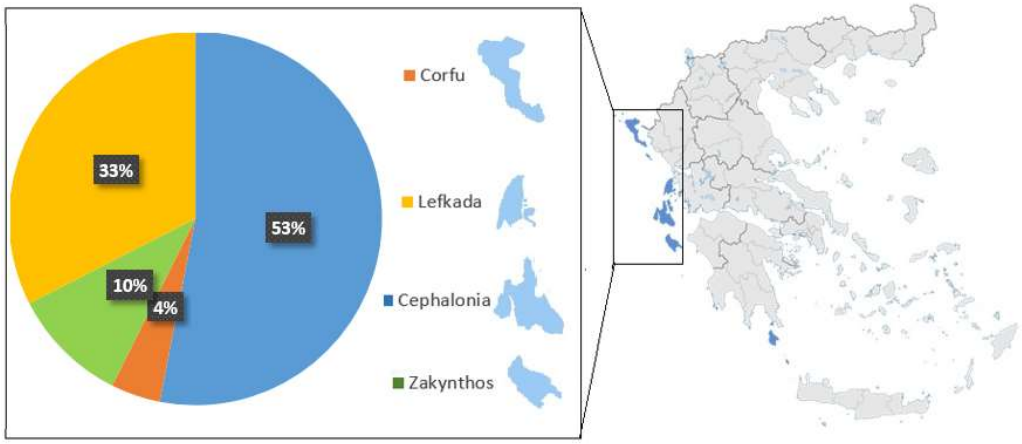

Figure 5. Spatial distribution of winery by-products in Ionian Islands.

5. Conclusions

The present work outlined the importance of qualitative characteristics of winery by products especially from traditional PDO grape varieties of the Ionian Islands as a critical input to any waste management plan towards circular bioeconomy. The waste samples were collected directly from the wineries immediately after the vinification process in order to give a realistic dimension. Additionally, the solvents were used were non-toxic and environmentally friendly. Furthermore, the application of green extraction processes enables the final products be safety for human thus increasing the breadth of demand. Grape pomace can be used to empower business sectors of the food industry, beverage, medicine, cosmetics, cooking, feed and many others. Also, with a short preprocessing, includes moisture removal and pulverization, it can be used as a whole material. Furthermore, the same handling results in reducing the environmental and economic costs due to their easiest and fastest transporting. All the above confirms the definition of the circular economy that something previously considered as "waste", can now be converted into raw material. The qualitative data of winery byproducts, combined with production data by variety and per island on an annual basis, allowed for a first mass balance estimation that could form the basis of any management plan after a feasibility study. In any case, further study is necessary on the optimization of each processing step to maximize the performance.

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