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Soil management affects the nutraceutical properties of Primitivo's grape pomace

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Abstract. Soil management is a sustainable agronomic practice to produce grapes, wine, and grape pomace with a low environmental impact in viticulture, affecting soil microbial biodiversity, organic matter, and healthy roots. Grape pomace is the main by-product of winemaking and a valuable source of natural phytochemicals. This research aimed to evaluate the phenolic content and antioxidant activity of pomace deriving from the microvinification of the Primitivo wine grapes obtained by four different soil management techniques: cover crop (C), soil mechanical tillage (T), green manure (G) and farm soil management (F). The content of total phenolic compounds and anthocyanins in grapes and wines is the highest with the vineyard cover cropping system. Moreover, grape pomace derived by cover crop soil management shows a significant abundance of these molecules and a more elevated antioxidant activity than the other soil technique.

1 Introduction

Sustainable agriculture is a broadly defined goal, and many organic growing practices can be used to fulfil this goal. The management techniques used in sustainable agriculture differs from conventional methods because to encourage beneficial insects, soil improvement through regular additions of organic matter, and significantly reducing the use of agrochemical input, improving the vineyard ecosystem. Sustainable grape-growing focuses on producing grapes that have minimal effects on the environment and are ecologically sound [1] and also wines more sustainable.

The winemaking process generates, during all its stages, an ample variety of solid and liquid by-products such as vine shoots, grape marc or grape pomace, wine lees, spent filter cakes, vinasses, and winery wastewater that must be treated, disposed of, or reused correctly to avoid negative environmental impacts [2,3].

Grape pomace represents an economic and environmental problem. It consists of water (~50%), neutral polysaccharides (~20%), pectic substances (~20%), insoluble proanthocyanidins, lignin, structural proteins, and phenols (~15%) [4].

The pomace is rich in phenolic compounds such as, anthocyanins, flavones, tannins and resveratrol, molecules with significant antioxidant potential [5,6].

Immediately after their production, pomace is rich in water, compromising its chemical stability and favouring deterioration by microbes. So, it is essential to reduce the water content of pomace to slow down these processes [7].

Pomace composition depends on the *terroir*, grape variety, degree of grape ripeness and the type of winemaking practices to produce red, rosé, or white wines. [8,9]. According to assessments, 1 kg of grape pomace is generated for each 6 L of wine [10]. Grape pomace represents the residue of the fresh grapes pressing process and can be fermented.

Grape pomace is one of the most important residues of the wine industry and constitutes about 20-25% of the total processed grapes [11]. The waste disposal, in high quantities, especially during the harvest season, creates groundwater and surface water pollution, attracts vectors that spread diseases and generates oxygen consumption with a significant impact on wildlife [12]. Furthermore, adverse effects are also found in the natural biodegradation processes due to the low pH and antibacterial substances [13].

On the other hand, grape pomace contains many beneficial molecules, such as polyphenols, which even remain after the winemaking process (about 70%) [14,15].

During the winemaking process, tons of pomace are obtained, consisting of skins, seeds, and stems. These components, especially the seeds, are rich in phenolic compounds with potent antioxidant, anticancer, anti-ageing, antimicrobial and anti-inflammatory properties [16].

In recent years, winemaking and agricultural by-products of vegetable origin attracted considerable attention due to their bioactive compounds, used in the cosmetic, pharmaceutical and food fields [17].

Therefore, special attention has been given to more profitable and sustainable options by the scientific community and producers aiming to utilize better all raw materials and by-products derived from the wine industry to reduce waste disposal to a minimum [18].

This research aimed to evaluate the phenolic content and antioxidant activity of pomace deriving from the microvinification of Primitivo wine grapes obtained by four different soil management techniques. The three inter-row soil management were compared: cover crop (C), soil mechanical tillage (T), green manure (G) and farm soil management (F).

2 Materials and methods

2.1 Plant materials, berries sampling and vinification

The research was carried out during the 2022 season in a five-year-old commercial vineyard located in the Castel del Monte Denomination of Controlled and Guaranteed Origin (D.O.C.G.) wine area in the Apulia region, Southern Italy, near Corato (lat. 41°05′36″ N, long. 16°28′22″ E, 354 m above sea level) on a shallow, gravel, sandy-loam soil, with sub-alkaline reaction and high organic matter content. *Vitis vinifera* L. ‘Primitivo’ variety, grafted onto *Vitis berlandieri* × *Vitis rupestris* 775 Paulsen rootstocks, were planted in north-south oriented rows. Vines were spaced 2.3 m between rows and 1.10 m on the row, trained to the Vertical Shoot Positioned (VSP) system and cane pruned with 14 buds per vine. The area’s climate is ‘Mediterranean’, with the coldest month mean temperature of 3 °C (January), the hottest month mean temperature of 33 °C (August) and 500 mm average rainfall per year, mainly concentrated from September to April. Under such high environmental evaporative demand and low and irregular rainfall, irrigation becomes necessary for wine grape production. Four inter-row soil management were compared: mechanical cultivation along the rows while inter-rows space was occupied by cover crop such as permanent resident vegetation. (C), inter-row soil mechanical cultivation along the rows and inter-rows (T), sowing of the inter-row with a mixture of *Brassicaceae* (Nematex BN1, De Corato Sementi, Andria, Italy) and subsequent green manure in the phase of maximum biomass production at the end of April (G) and farmer soil management (inter-row cover crop alternate to tillage inter-row (F). In conclusion, in a natural cover crop, vineyard soil management consists of two zones: the rows, a 70 cm-wide area underneath the vines, which are managed primarily to control weeds by mechanical cultivation, and the middles interspersed between the rows, which are vegetated by resident vegetation and are mown two times per year in spring and early summer. Cultivation was carried out every 4 to 5 weeks during the growing season with a cultivator equipped with a trunk sensor to avoid vines damage. The Experimental plot was designed in four blocks of vines, each consisting of 4 rows for 250 vines.

Harvest was performed manually, and grape composition (total soluble solids, titratable acidity, and pH) was also determined (Table 1). Besides, about 80 kg of grapes of both soil management treatments were wine processed in the experimental winery of the Research Centre for Viticulture and Enology, according to a specific protocol [19]. For phenolic evaluation, 30 different berries were randomly collected from five bunches of grapes and immediately stored at -20 °C until use. For each sample, three biological replicates were taken.

2.2 Grape pomace extract (GPE) preparation

Ten days of maceration were applied with two punch-downs per day. When maceration was concluded, free-run wine was recovered, pomace was gently pressed to obtain press-run wine, and pomace was successfully collected. After preliminary tests at different drying temperatures and times, the grape pomace was dried at 40° for 48 h in a ventilated oven. Then, the grape pomace was ground by an electric mill. For extract preparation, 0,5 g of dried powder was resuspended in 10 mL of ethanol solution: water: hydrogen chloride 37% (70:30:1 v/v/v). After 24 hours under dark conditions, the mixture was filtered through a 0.45 μm syringe cellulose filter and immediately analyzed or stored at -20 °C.

2.3 Determination of total phenolic content (TPC) and total anthocyanins (TA)

TPC was determined using the microscale protocol Waterhouse (2009) [20] described. Briefly, 1 mL of water, 0.02 mL of extract sample, 0.2 mL of the Folin-Ciocalteu reagent, and 0.8 mL of 10% sodium carbonate solution were mixed and brought to 3 mL. The absorbance was measured at 760 nm after 90 min at room temperature with a spectrophotometer Agilent 8453 (Agilent Technologies, Santa Clara, CA). Results were expressed as milligrams of gallic acid equivalent/g of dry weight based on a gallic acid calibration curve (50 to 500 mg/ with $R2 = 0.998$). TA was determined using a pH differential protocol proposed by Lee et al. (2005). Appropriate grape extract dilutions were mixed with I 0.025M potassium chloride (pH 1) or 0.4M sodium

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acetate (pH 4.5) buffers. Absorbance was measured at 520 and 700nm with the spectrophotometer system Agilent 8453 (Agilent Technologies, Santa Clara, CA). Results were expressed as milligram cyanidin 3-glucoside equivalents per gram of grape skin (mg Cy/g skin).

2.4 Antioxidant activity

The antioxidant activity was evaluated by radical scavenging assays based on two different tests: DPPH assays and ORAC assay were performed. Calibration curves were prepared using Trolox, and values were expressed as mM TE/Kg dried pomace.

The DPPH (2,2 O-diphenyl-1-picrylhydrazyl) assay was conducted according to Brand-Williams et al. (1995) [21] technique with some modifications. The stock solution was prepared by mixing 2.5 mg DPPH radical with 100 mL ethanol. The solution absorbance was adjusted at 0.7 ± 0.02 in 515 nm using a UV–Vis spectrophotometer Agilent 8453 (Agilent Technologies, Santa Clara, CA). 2mL of DPPH radicals was mixed with 200 µL of the sample extract or standard (ethanol was used as blank). The decrease in absorbance at 515 nm was measured after 30 min of incubation at 37 °C.

Oxygen Radical Absorbance Capacity (ORAC) assay was performed as previously reported [22]. Briefly, ORAC analysis was carried out using a plate reader FLUOstar OPTIMA (B.M.G. Labtech, Germany), fluorescein as a probe with an excitation wavelength of 485 nm, and an emission wavelength of 520nm. The fluorescence was measured every 2 min for 120 min at 37 °C The final ORAC values were calculated using the area differences under the fluorescence decay curve (AUC) between the blank and the sample.

2.5 Statistical analysis

All measurements were carried out in triplicate, and the results were analysed statistically using STATGRAPHICS Centurion software (Stat-Ease, Minneanopolis, MN, USA). One-way analysis of variance (ANOVA) was used to determine the effect of soil management on the nutraceutical properties of Primitivo's grapes, wines, and pomace. Multiple Range Tests was applied to determine the significance of differences between means, and the statistical significance was set at $p < 0.05$.

3 Results

3.1 Berry composition

Table 1 shows the pH, titratable acidity (TiA) and the total soluble solid (TSS) concentration at harvest. The same pH and TiA values were recorded in all four treatments. As TSS, the cover crop management caused a statistically significant increase in TSS.

Table 1. Berry composition of Primitivo grapes.

Treatment	pH	Total acidity (g/L)	Total soluble solids (° Brix)
Green manure	3.55	5.73	21.23 ab
Tillage	3.51	6.55	21.73 ab
Cover crop	3.67	6.09	24.13 a
Farm soil management	3.49	6.04	19.63 b

In columns, different letters indicate statistically significant differences at $p < 0.05$.

3.2 Total phenolic content and total anthocyanin content of Primitivo grapes and wines

Total phenolic and anthocyanin content obtained from grapes and wine characterization is shown in Table 2.

First, TPC and TA of the grapes obtained by the four-soil management were determined. The cover crop treatment showed higher levels of TPC and TA (1015 mg/Kg GAE and 281 mg/kg Cyd-3-gluc, respectively), followed by green manure management. In the wine evaluation, the highest values of TPC and TA were measured in cover crop treatment (1733 mg/Kg GAE and 136 mg/kg Cyd-3-gluc, respectively), followed by tillage management (1592 mg/Kg GAE and 105 mg/kg Cyd-3-gluc, respectively).

Table 2. Total phenolic content and total anthocyanin content of Primitivo grapes and wines.

Grapes		
Treatment	**TPC** (mg/Kg fresh grape GAE)	**TA** (mg/kg Cyd-3-gluc fresh grape)
Green manure	881 ab	196 ab
Tillage	798 b	140 b
Cover crop	1105 a	281 a
Farm soil management	659 b	110 b
Wines		
Treatment	**TPC** (mg/L GAE)	**TA** (mg/L Cyd-3-gluc)
Green manure	1367 c	126 c
Tillage	1592 b	105 b
Cover crop	1733 a	136 a
Farm soil management	1339 c	107 c

In columns, different letters indicate statistically significant differences at $p < 0.05$.

3.3 Total phenolic content, total anthocyanin content and antioxidant activity of Primitivo pomace

Table 3 shows Primitivo grape pomace's TPC and TA.

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As grapes and wine, the highest content of phenolic compounds was observed in cover crop treatment (44211 mg/Kg dried pomace GAE).

Regarding TA, both cover crop and farm soil management caused the more significant accumulation of these bioactive compounds.

Table 3. Total phenolic content and total anthocyanin content of Primitivo grape pomace.

Treatment	TPC (mg/Kg dried pomace GAE)	TA (mg/kg Cyd-3-gluc dried pomace)
Green manure	39411b	1823 b
Tillage	40791 b	1728 b
Cover crop	44211 a	2116 a
Farm soil management	39595 b	2193 a

In columns, different letters indicate statistically significant differences at $p < 0.05$.

More than one type of antioxidant capacity measurement needs to be performed to consider the different ways of acting by antioxidants: assays based on hydrogen atom transfer (HAT-based assays), such as ORAC, and assays based on electron transfer (ET-based assays), such as DPPH. Table 4 shows that the grape pomace obtained by tillage management possesses the highest value of DPPH. The cover crop management causes the highest antioxidant activity ORAC value in grape pomace.

Table 4. Antioxidant activity of Primitivo grape pomace

Treatment	DPPH (mM TE/kG dried grape)	ORAC (mM TE/kG dried grape)
Green manure	644,29 b	258,84 b
Tillage	654,36 a	264.57 b
Cover crop	642,48 b	350,83 a
Farm soil management	623,52 c	216.37 b

In columns, different letters indicate statistically significant differences at $p < 0.05$.

4 Discussion

Grape pomace composition highly depends on wine grape variety, canopy microclimate, method, and many other factors. One of the most critical factors influencing wine phenolic composition is grape variety. Other factors to consider are the variability of climatic conditions of each growing season, cluster exposure to sunlight, berry maturity degrees and vineyard management, such as soil management with cover crops or the foliar application of nitrogen compounds [23].

Pomace contains many beneficial molecules, such as polyphenols, with various functional groups in their structures. Phenolics are secondary metabolites produced by plants in response to biotic or abiotic stresses, with an important role in berry skin color, flavor, the taste of wine. Phenolic compounds in grapes can be divided into these categories according to their structure and degree of polymerization: phenolic acids (hydroxybenzoic and 00 hydroxy-cinnamic acids), simple flavonoids (flavan-3-ols, flavonols, and anthocyanins), a higher degree of polymerization flavonoids (proanthocyanidins/ condensed tannins) and stilbene (resveratrol) [24]. They can perform various biological activities [25] such as maintaining intestinal health and preventing chronic diseases and cancer.

Many studies showed the great antioxidant potential of polyphenols due to the inhibition of lipid oxidation. Their antioxidant activity is based on different mechanisms, such as the radical scavenging ability, electron donation, or chelation of metal ions [26,27]. It depends on their structure, particularly on the number and position of the hydroxyl groups and the nature of the substitutions on the aromatic rings [28].

Our study evaluated the phenolic content and antioxidant activity of grape pomace deriving from the microvinification of Primitivo wine grapes obtained by sustainable soil management techniques. The results showed that the grape pomaces are sources rich in phenolic and antioxidant molecules. Although some classes of polyphenols and phenolic acids are under strict genetic control, the final content is highly influenced by environmental factors, such as climate, soil, vineyard, and management [29].

Specifically, the inter-row cover cropping system increases the content of total phenolic compounds and total anthocyanins in Primitivo grapes and wines. This increase was also evident in Primitivo grape pomace, which shows a significant abundance of these molecules in cover crop soil management. In further support of this data, the antioxidant activity of Primitivo grape pomace was evaluated by radical scavenging assays based on DPPH and ORAC tests. These tests highlighted how cover crop soil management helps improve the antioxidant power of Primitivo grape pomace (Table 4).

The significant increase in phenolic content agrees with the results of other researchers [30,31]. Other Authors reported the same effect on the phenolic content because of cover crop competition for water and nitrogen. This represents a stress for the plant with consequences on phenolic metabolism, grape development, and chemical composition [32]. Cover crop soil management causes an increase in water and nutrient consumption and, consequently a reduction in grapevine vegetative growth. This represents an advantage for grape health and berry composition. It induces a more favourable balance between vegetative and reproductive growth. It allows a more open canopy and, consequently a better microclimate in the cluster zone, improving fruit color and total phenols and anthocyanin content in grapes [33-38].

5 Conclusions

Grape pomace, a byproduct accumulated mainly during wine production, represents a valuable source of essential nutrients and bioactive compounds. As this work demonstrated, the evaluation of phenolic compounds and antioxidant activity of Primitivo grape pomace indicates the positive impact of soil management on its nutraceutical properties.

This study on Primitivo grape pomace, concerning different soil management techniques, showed that inter-row cover crop can increase pomace's total phenolics and its potential as a source of healthy substances.

Using grape pomace as a source of healthy substances is a promising field. In this way, the recovery of bioactive compounds from grape pomace can still be an attractive field of waste generation and environmental approach.

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