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Health benefits of winemaking by-products: *in vitro* study of the phenolic profile and potential healthy properties

Corinne Bani[1], Patrizia Restani[1], Antonella Bosso[2], Francesca Mercogliano[1] and Chiara Di Lorenzo[1]

[1] Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy
[2] Consiglio per la Ricerca e Sperimentazione in Agricoltura - Centro di Ricerca per l'Enologia, Asti, Italy

Abstract. The wine industry generates great amounts of by-products, with grape pomace being one of the most abundant. Grape pomace, traditionally used to produce distillates, fertilizers and animal feed, is finding new interest thanks to its nutritional and health advantages. The study aimed at analyzing the phenolic composition and antioxidant capacity of grape pomace from different *Vitis vinifera* L. varieties and to investigate its impact on enzymes related to glucose metabolism and glycemic regulation. Different in vitro methods were used to measure different parameters: Folin-Ciocalteu assay for total polyphenol content; differential pH test for total anthocyanin content; DPPH assay, FRAP assay and HPS assay for antioxidant activity. The extracts were also tested for their inhibitory effects on dipeptidyl peptidase IV (DPP-IV), pancreatic α-amylase, and α-glucosidase. The spectrophotometric analysis offered valuable insights into phenolic composition, revealing a strong correlation with antioxidant capacity. Grape pomace, especially when including seeds, emerged as a potent source of polyphenols with notable antioxidant properties and the ability to potentially inhibit enzymes involved in postprandial blood sugar regulation. These results indicate that winemaking by-products, such as grape pomace, could serve as important source of bioactive compounds to be included in dietary supplements and other functional products, promoting sustainability in wine industry.

1. Introduction

The wine sector holds a relevant position in the international agri-food industry, contributing significantly to economy, culture, and heritage of many regions across the globe. Based on information collected in twenty-nine countries (94% of global production in 2022) global wine production (excluding juices and musts) is estimated between 241.7 mhL and 246.6 mhL in 2023 [1]. Wine industry is characterized by a complex and extensive wine production process that inevitably leads to the generation of massive amounts of by-products [2]. Among these by-products, grape pomace stands out as one of the most abundant [3]. Pomace, which consists of the skins, seeds, and stems remaining after the extraction of grape juice during winemaking, has traditionally been utilized in various applications such as the production of distillates, fertilizers, and animal feed [4]. Despite these uses, a significant portion of grape pomace often remains underutilized, representing a lost opportunity for adding value to the winemaking process. In recent years, there has been a growing shift towards the adoption of circular economy models within the wine industry, driven by the increasing emphasis on sustainability and waste reduction. These models aim to extend the lifecycle of products and valorise food waste and by-products, thereby minimizing environmental impact and promoting resource efficiency [5]. In this context, grape pomace is attracting significant attention due to its rich nutritional properties and potential health benefits. Grape pomace is recognized as a valuable source of functional compounds, particularly polyphenols, which have been widely studied for their antioxidant and other health-promoting properties [6]. These compounds are increasingly being explored for their applications in food, feed, and cosmetic formulations, offering a promising alternative for the valorisation of this abundant by-product. Despite the extensive scientific literature that highlights the beneficial effects of polyphenols on cardiovascular functions and oxidative stress, there remains a notable gap in understanding their role in modulating postprandial glycemia. Postprandial glycemia - the blood sugar level following a meal - is a critical factor in the management of diabetes (the major health problems worldwide) and other metabolic disorders [7]. The potential role of grape pomace polyphenols in influencing enzymes involved in glucose metabolism, could open new pathways for the development of functional foods and nutraceuticals aimed at glycemic control. The primary

objective of this study was to compare various in vitro methods used to evaluate the phenolic profile and antioxidant capacity of grape pomace derived from different *Vitis vinifera* L. varieties. Additionally, the study aimed to investigate the efficacy of these extracts in modulating the activity of enzymes that play crucial roles in glucose metabolism and glycemic regulation. Particularly, the inhibitory activity on dipeptidyl peptidase IV (DPP-IV), α-amylase and α-glucosidase was evaluated. The dipeptidyl peptidase IV (DPP-IV) enzyme breaks down and inactivates glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which are gut incretin hormones secreted in response to nutrients. These hormones stimulate glucose-dependent insulin secretion in pancreatic beta cells. Inhibition of DPP-IV has been shown to extend the half-life of circulating GLP-1, reduce plasma glucose levels, and improve glucose tolerance [21]. Additionally, glucose homeostasis control can also be achieved by inhibiting carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase. Inhibitors of salivary and pancreatic α-amylase prevent the hydrolysis of complex starches into oligosaccharides, thereby reducing glucose absorption. Similarly, α-glucosidase inhibitors block the breakdown of di-, tri-, and oligosaccharides into glucose in the small intestine, resulting in decreased carbohydrate digestion and reduced glucose absorption into the bloodstream [21]. By elucidating the bioactive potential of grape pomace, this research tries to contribute to the development of innovative, health-promoting products and support sustainable practices within the wine industry. This approach not only enhances the value of winemaking by-products but also aligns with broader efforts to promote environmental sustainability and improve human health through the utilization of natural resources.

2. Materials and methods

2.1. Samples

The ten samples of winemaking by-products were from red grapes and were provided by Dr. Antonella Bosso (Viticulture and Oenology Research Center, CREA, Asti, Italy). As shown in **Table 1**, samples included in the study were obtained from grapes of different vintages and types; furthermore, products deriving from different phases of winemaking were compared.

Table 1. Samples of grape pomace from various *Vitis vinifera* L. varieties, collected at different winemaking stages in different years.

Samples		Winemaking stage	CODE
Grignolino, 2015	Seeds	Initial fermentation	G-IF-S-15
Grignolino, 2015	Seeds	After fermentation	G-AF-S-15
Grignolino, 2016	Seeds	Initial fermentation	G-IF-S-16
Grignolino, 2016	Seeds + skins	After fermentation	G-AF-SS-16
Grignolino, 2018	Seeds + skins	After fermentation	G-AF-SS-18
Uvalino, 2015	Seeds	Initial fermentation	U-IF-S-15
Uvalino, 2015	Seeds + skins	After fermentation	U-AF-SS-15
Uvalino, 2016	Seeds + skins	After fermentation	U-AF-SS-16
Barbera, 2017	Seeds + skins	After fermentation	B-AF-SS-17
Barbera, 2018	Seeds + skins	After fermentation	B-AF-SS-18

After grinding with Mulinex illico, the samples were stored at a temperature of -20°C until analysis.

2.2. Phenolic profile assays

The characterization of soluble phenolic compounds and anthocyanins of the samples has been carried out and described in a previous paper by our group [3,8].

2.2.1. Folin-Ciocalteu assay

The soluble polyphenol content was determined using the Folin-Ciocalteu method, as described by Singleton et al. and in our previous papers [8, 9]. Briefly, 0.4 g of ground grape pomace, 3 mL of a methanol:water 1:1 (v/v) hydroalcoholic mixture were added. The samples were placed in an ultrasonic bath for 15 minutes and then centrifuged at 8000 rpm, at 4°C for 15 minutes. After centrifugation, the supernatant was collected and filtered using Whatman Grade 1 paper filters. To the residue, 2 mL of the methanol:water 1:1 (v/v) mixture was added, and sonication, centrifugation, and filtration steps were

repeated under the same conditions. The supernatants were combined and brought to volume with the methanol:water 1:1 (v/v) solution. For the subsequent analyses, sample solutions were appropriately diluted using the same extraction solvent. Each sample was extracted in triplicate. In test tubes, 300 µL aliquots from various suitably diluted samples (or water for the blank) were combined with 1.5 mL of 0.2 N Folin-Ciocalteu reagent and 1.2 mL of 7.5% sodium carbonate. The mixture was left in the dark for 30 minutes before measuring the absorbance at 765 nm using a UV-visible spectrophotometer (Varian Cary 50 SCAN, Palo Alto, CA, USA). The results were expressed in mg/g as gallic acid equivalents (GAE). A standard curve of gallic acid, ranging from 5 to 50 mg/mL, was used to calculate the polyphenol content in the samples. Each analysis was conducted in triplicate.

2.2.2. Total Anthocyanin Content

The total anthocyanin content in samples was determined by spectrophotometric analysis using the AOAC method, which is based on the pH differential assay [10]. The extraction of anthocyanins is typically conducted using methanol or ethanol acidified with 1 M HCl to obtain the aglycone in the form of a flavylium cation, which is highly stable in an acidic environment [11]. Approximately 0.4 g of each sample was combined with 3 mL of an methanol:1M hydrochloric acid (85:15, v/v). The mixture was sonicated for 15 minutes in an ultrasonic bath, followed by centrifugation for 15 minutes at 8000 r.c.f. (relative centrifugal force) at 4 °C. After centrifugation, the supernatant was filtered through a 0.45 µm polypropylene filter and collected. A second extraction was carried out on the solid precipitate, and the two resulting supernatants were combined. The combined solution was adjusted to a final volume of 5 mL using methanol:1M hydrochloric acid (85:15, v/v). Samples were suitably diluted with KCl buffer (0.025M potassium chloride, pH 1) and CH_3COONa buffer (0.4M sodium acetate, pH 4.5) and their absorbance was measured at 520 nm and 700 nm, with the latter reading used for haze correction. Each analysis was conducted in triplicate. The total anthocyanin content (TAC) was quantified as cyanidin-3-*O*-glucoside equivalents (CY mg/g) using the following equation (1):

$$AP\ (CY\ mg/g)=\Delta A\times MW\times DF\times 1000\times V/e\times l\times W$$

In this equation: ΔA is the difference between ($A_{520\ nm} - A_{700\ nm}$) at pH 1.0 and ($A_{520\ nm} - A_{700\ nm}$) at pH 4.5; MW is the molecular weight of cyanidin-3-*O*-glucoside (449.2 g/mol); DF is the dilution factor; 1000 is the conversion factor from g to mg; V is the extraction volume; e is the molar extinction coefficient for cyanidin-3-*O*-glucoside (26,900); l is the path length in centimeters (1 cm); and W is the sample weight. Since anthocyanins are contained only in the grape skin of red varieties [12], this method was applied only for the analysis of the following samples: G-AF-SS-16, G-AF-SS-18, U-AF-SS-15, U-AF-SS-16, B-AF-SS-17, B-AF-SS-18.

2.3. Anti-oxidant activity assay

In vitro antioxidant activity (AOA) is one of the functional properties most extensively investigated, and several assays have been developed for the in vitro evaluation of AOA; to achieve a comprehensive assessment, it is essential to apply different methods in parallel [13]. Three different spectrophotometric methods were used to evaluate the in vitro antioxidant activity of the samples: the 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) assay, Ferric Reducing Antioxidant Power (FRAP) assay, Hydrogen Peroxide Scavenging (HPS) assay. The antioxidant activity of samples measured by DPPH and FRAP assay has been carried out and published in our previous papers [3,8].

2.3.1. DPPH assay

The antioxidant capacity of samples was evaluated as a measure of radical scavenging activity using the DPPH spectrophotometric assay [14,15]. The sample extraction was performed following the procedure applied and described in Paragraph 2.2.1 [8]. Gallic acid (GA) standard solutions were prepared in a mixture of methanol and water (1:1 v/v) (range of 1-5 µg/mL). Then, aliquots of 1 mL DPPH solution (Sigma Aldrich, Germany), dissolved in methanol at a concentration of 5 mg/100 mL, were combined with 0.5 mL of standard solution or samples. Subsequently, the absorbances were measured at 517 nm after a 30-minute incubation period in the dark using a UV-visible spectrophotometer (Varian Cary 50 SCAN, Palo Alto, CA, USA). Results were then quantified in terms of gallic acid equivalents (GAE), expressed as milligrams per gram of the sample (mg GAE/g).

2.3.2. FRAP assay

In the Ferric Reducing Antioxidant Power (FRAP) assay, the oxidized and colorless form of iron, Fe^{3+}, is converted by antioxidant compounds into its reduced form, Fe^{2+}. In presence of 2,4,6-tripyridyl-s-triazine (TPTZ), a blue-colored TPTZ-Fe^{2+} complex is formed, which shows a characteristic absorption peak at 593 nm [16,17]. Samples were prepared following the procedure described in section 2.2.1 [8]. The antioxidant capacity was determined using a standard curve of ferrous sulfate heptahydrate (Sigma Aldrich, Germany) with concentrations ranging from 0.11 to 0.75 mM. The FRAP reagent was previously prepared by combining 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution, and 20 mM $FeCl_3*6H_2O$ (iron chloride hexahydrate) solution in a ratio of 10:1:1 (v/v/v). Volumes of 50 µL of standard solution, sample (appropriately diluted), or blank (methanol:water 50:50, v/v) were mixed with 150 µL of HPLC grade water and 1500 µL of FRAP reagent; then, were incubated at 37 °C for 30 minutes in the darkness. Subsequently, the absorbance was measured at 593 nm using a UV-visible spectrophotometer (Varian Cary 50 SCAN, Palo Alto, CA, USA). The results were expressed as millimoles (mmol) equivalent of Fe^{2+} (EFe^{2+})/g of sample [8].

2.3.3. HPS assay

The ability of plant extracts to neutralize the effect of hydrogen peroxide (H_2O_2) can be estimated according to the Hydrogen Peroxide Scavenging (HPS) method of Ruch and collaborators (1989) [18]. The sample extraction was performed using only methanol following the procedure applied and described in Paragraph 2.2.1. The antioxidant capacity was determined using a standard curve of ascorbic acid (Sigma Aldrich, Germany) ranging from 25 to 400 µg/mL. Four solutions were prepared: 1) Buffer: 1.5 mL of 50 mM Phosphate Buffer (PB) 2) Blank: add 1 mL of PB to 0.5 mL of standard or sample. 3) Test: add 1 mL of 40 mM H_2O_2 Reagent (RX) to 0.5 mL of standard or sample 4) Control: add 1 mL of RX to 0.5 mL of solvent used for extraction. After mixing for 10 min in the dark, the absorbances were measured at 230 nm using a UV-visible spectrophotometer (Varian Cary 50 SCAN, Palo Alto, CA, USA). The percentage of inhibition is calculated as follows:

$$\% \text{ scavenger } (H_2O_2): (Ai - At/Ai) \times 100\%$$

Where: Ai is the absorbance of the control and At is the absorbance of the test sample. Antioxidant capacity is expressed as mg of ascorbic acid equivalents (EAA) relative to dry sample weight (g).

2.4. In vitro assays for DPP-IV, α–amylase, and α–glucosidase inhibitory activities

2.4.1. Sample preparation

About 1 g of the samples were suspended in 10 mL of ethanol:water (60:40, v/v) [19] and stirred for 2 hours at room temperature in the dark. After extraction, the samples were centrifuged at 2000 x g for 15 minutes at 4°C (5810-R, Eppendorf, Hamburg, Germany) and filtered through a Whatman grade 1 paper filter. The extracted solutions were stored at -20°C until further analysis. Each sample was extracted in triplicate. All samples were analyzed at a concentration of 5 mg/mL.

2.4.2. DPP-IV inhibitory activity

Dipeptidyl peptidase IV (DPP-IV) inhibitory activity was measured in 96-well plates following the protocol described by Silveira et al. (2013) [20]. Recombinant soluble human DPP-IV (15 µL) was incubated with samples (10 µL) and different volumes of buffer Tris HCl 50mM pH 7.5 for 10 minutes. Diprotin A served as the positive control. After the incubation period, 50 µL of the substrate containing H-Gly-Pro-p-nitroaniline was added to each well. Absorbance was measured at 37°C at 405 nm using a Enspire® Multimode plate reader (PerkinElmer, Waltham, MA, USA) at 2-minute intervals, starting from 0 up to 30 minutes. The data were then expressed as the percentage of remaining enzyme activity in the presence of test samples compared to the control [21]. Each sample was analyzed in triplicate.

2.4.3. α–amylase inhibitory activity

The α-amylase inhibition assay was adapted from a method by Vilcacundo et al., 2017 [21]. Firstly, 50 µL of the sample, positive control (2 mM acarbose), or negative control (20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9) were added with 100 µL of α-amylase solution (2 U/mL in Tris-HCl buffer, pH 7.5, 100 mM KCl). The test tubes were then incubated at 20°C 1000 rpm for 5 minutes. Next, 100 µL of a 1% soluble starch solution (dissolved in 20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9, and boiled for 15 minutes) were added to each tube and incubated at 20°C and 1000 rpm for 6 minutes. After this incubation, 100 µL of dinitrosalicylic acid color reagent was added, and after a gentle shake, they were placed in a 100°C water bath for 15 minutes. Then, 800 µL of distilled water were added to the mixture; the absorbance was measured at 540 nm using an Enspire® Multimode plate reader (PerkinElmer, Waltham, MA, USA). Maltose concentration was calculated using a linear standard curve (0–2.23 mg/mL) generated from a freshly prepared stock solution 0.2%. Percent of inhibition was calculated in comparison to the negative control (100% enzyme activity).

2.4.4. α–glucosidase inhibitory activity

The α-glucosidase inhibition assay was performed according to the method by Vilcacundo et al., (2017) [21] with some modifications. Firstly, 100 µL of the sample, positive control (1 mM acarbose), or negative control (0.1 M maleate buffer, pH 6.9) were added with 50 µL of rat intestine α-glucosidase (1 U/mL in 0.1 M maleate buffer, pH 6.9). The tubes were incubated at 37°C for 5 minutes 1000 rpm. After pre-incubation, 50 µL of the substrate (2 mM maltose) were added to each tube. The reaction mixtures were then incubated at 37°C for 30 minutes 1000 rpm. The reactions were stopped by placing the tubes in a 100°C water bath for 5 minutes. The supernatants were collected by centrifugation at 10000 rpm for 5 minutes at 20°C and then stored at -20°C. The glucose concentration in the reaction mixtures was determined using the total starch (100A) assay kit (Megazyme, Irland). Absorbance was measured at 510 nm using an Enspire® Multimode plate reader (PerkinElmer, Waltham, MA, USA). Glucose concentration was calculated using a linear standard curve (0–4 mM) generated from a freshly prepared 400 mM stock solution. Inhibition was calculated as a percentage of the negative control activity, which was considered equivalent to 100%.

2.5. Statistical analysis

Data were subjected to ANOVA one-way analysis using the Duncan test with the SPSS - Statistics for Campus-Pro Premium V.29 for Macintosh.

3. Results and discussion

3.1. Assessment of phenolic profile

Figure 1 shows the result obtained from the quantification of the total polyphenol content (TPC) and the total anthocyanin content (TAC) of all samples included in the study [3,8].

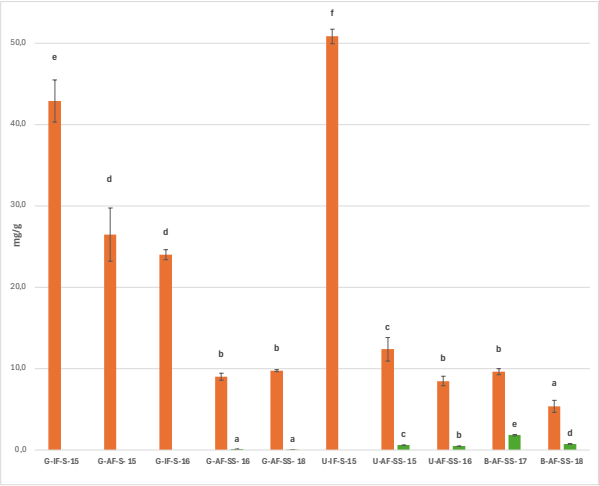

Figure 1. Total polyphenol content (TPC, orange bar, mg/g of gallic acid equivalents, mean±SD; n=3) and total anthocyanin content (TAC, green bar, mg/g of cyanidin-3-*O*-glucoside equivalents, mean±SD; n=3) of winemaking by-products containing skins. For the same column, samples having different lower-case letters have statistically significant difference ($p < 0.05$).

Based on data published in our previous papers by Di Lorenzo et al., 2023 [3, 8], TPC varied significantly among different grape samples. Uvalino seeds (U-IF-S-15) showed the highest TPC (50.8±0.9 mg GAE/g), while Barbera skins and seeds (B-AF-SS-18) the lowest (5.4±0.8 mg GAE/g). In Grignolino samples, TPC was higher at the beginning of fermentation period (G-IF-S-15, 42.9±2.6 mg GAE/g) compared to the same samples collected in post-fermentation stage (G-AF-S-15, 26.5±3.3 mg GAE/g), showing a 38% reduction due to by-products from racking. Total Anthocyanin Content (TAC) also varied, with the highest content found in Barbera by-products from 2017 (B-AF-SS-17, 1.82±0.09 mg/g) and the lowest in Grignolino post-fermentation samples from 2018 (G-AF-SS-18, 0.05±0.01 mg/g). The anthocyanin content in grapes of the same cultivar can vary across different years, primarily due to changes in climatic factors such as rainfall, temperature, and relative humidity. Additionally, certain winemaking techniques can influence the extraction of anthocyanins and the residual anthocyanin levels in by-products [22,23].

3.2. Assessment of *in vitro* antioxidant activity

The antioxidant activity of winemaking by-products was previously measured using DPPH and FRAP assays [3,8]. This study introduced the Hydrogen Peroxide Scavenging (HPS) assay to provide a more comprehensive antioxidant profile. **Table 2** shows the total antioxidant activity (AOA) measured by DPPH, FRAP and HPS assays.

Table 2. Total antioxidant activity (AOA), measured by DPPH, FRAP, HPS assays of the samples analyzed. Samples in the same column having different lowercase letters had statistically significant difference ($p < 0.05$).

CODE	AOA assay		
	DPPH mg GAE/g mean±SD	FRAP mmol Fe^{2+}E/g mean±SD	HPS mg EAA/g mean±SD
G-IF-S-15	34.5±1.4^{e}	0.57±0.04^{d}	371.1±46.6^{e}
G-AF-S-15	26.3±0.5^{d}	0.40±0.04^{c}	184.8±16.7^{c}
G-IF-S-16	17.7±2.0^{c}	0.37±0.02^{c}	262.5±31.9^{d}
G-AF-SS-16	5.2±0.2^{ab}	0.14±0.01^{b}	101.2±4.3^{b}
G-AF-SS-18	3.5±0.4^{a}	0.15±0.01^{b}	101.1±5.3^{b}
U-IF-S-15	42.0±3.4^{f}	0.72±0.08^{d}	503.8±51.9^{f}
U-AF-SS-15	7.5±0.2^{b}	0.18±0.01^{b}	93.7±3.8^{b}
U-AF-SS-16	4.1±0.2^{a}	0.12±0.01^{ab}	41.4±5.3^{a}
B-AF-SS-17	5.2±0.3^{ab}	0.14±0.02^{b}	78.8±9.1^{ab}
B-AF-SS-18	2.8±0.0^{a}	0.07±0.00^{a}	66.6±5.7^{ab}

The antioxidant activity measured by DPPH assay ranged from 42.0±3.4 to 2.8±0.0 mg GAE/g. Samples containing only seeds exhibited higher antioxidant activity than those consisting of both seeds and husks. Similarly, the antioxidant activity measured by the FRAP test was higher in seed samples (0.72±0.08 – 0.37±0.02 mmol Fe^{2+}E/g) compared to the seed+husks samples (0.18±0.01 – 0.07±0.00 mmol Fe^{2+}E/g). Likewise, the antioxidant activity measured by the HPS assay was greater in seed samples (503.8±51.9 – 184.8±16.7 mg EAA/g) than in the seed+husks samples (101.2±4.3 – 41.4±5.3 mg EAA/g). A decrease in antioxidant capacity by 24%, 30%, and 50% (measured by DPPH, FRAP, and HPS assays, respectively) was detected in G-IF-S-15 and G-AF-S-15 samples collected after winemaking. Notably, U-IF-S-15 showed the highest antioxidant activity, attributed to its high polyphenol content, which significantly contributes to antioxidant capacity [24].

Figure 2 illustrates the correlation between soluble polyphenol content and antioxidant activity.

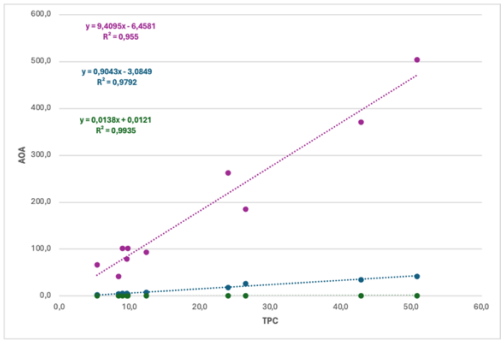

Figure 2. Correlation between soluble polyphenol content and antioxidant activity measured in by-product samples (blue line: DPPH assay; green line: FRAP assay; violet line: HPS assay).

As shown in **Figure 2**, a certain variability between samples was observed. The correlation between soluble polyphenol content and the antioxidant activity (measured by DPPH, FRAP and HPS methods), showed R^2 values of 0.9792, 0.9935, and 0.955, respectively. This indicates a good linear correlation; therefore, phenolic compounds are strictly correlated with the antioxidant activity of samples [24].

3.3. Assessment of *in vitro* glucose modulation

Dipeptidyl peptidase IV (DPP-IV) plays an important role in diabetes and autoimmune diseases. By inhibiting this enzyme, incretins such as GLP1 and GIP, involved in insulin production, can extend their action [25]. On the other hand, α-amylase cleaves the α-1,4-glycosidic bonds of amylose to produce maltose and glucose. α-amylase inhibition slows down the absorption of carbohydrates, influencing postprandial blood glucose and insulin levels, thus represents a strategy for managing type 2 diabetes [26,27]. Finally, α-glucosidase hydrolyzes the non-reducing terminal residues of α-glucose to release glucose molecules. α-amylase inhibitors slow down the digestion and absorption of carbohydrates, maintaining postprandial blood glucose at a lower level and reducing the demand for insulin [28]. Therefore, the inhibition of these enzymes is of great interest in developing supporting strategies to the therapeutic managing of type 2 diabetes mellitus. **Table 3** shows the result obtained by DPP-IV inhibitory activity assay, α-amylase inhibitory assay and α-glucosidase inhibitory assay. All samples were analyzed at a concentration of 5 mg/mL.

Table 3. Results obtained by enzymatic assays. Samples in the same column having different lowercase letters had statistically significant difference ($p < 0.05$).

CODE	Assay		
	DPP-IV Inhibition%	α -amylase Inhibition%	α -glucosidase Inhibition%
G-IF-S-15	70.4%^{d}	67.4%^{c}	N.D.
G-AF-S-15	31.2%^{a}	51.9%^{a}	N.D.
G-IF-S-16	72.4%^{d}	59.8%^{b}	N.D.
G-AF-SS-16	68.1%^{d}	84.3%^{g}	N.D.
G-AF-SS-18	70.3%^{d}	77.0%^{e}	N.D.
U-IF-S-15	56.3%^{bc}	89.1%^{h}	N.D.
U-AF-SS-15	57.2%^{c}	93.8%^{i}	N.D.
U-AF-SS-16	50.6%^{bc}	81.2%^{f}	N.D.
B-AF-SS-17	48.9%^{bc}	74.1%^{d}	N.D.
B-AF-SS-18	48.1%^{b}	73.3%^{d}	N.D.

The inhibition data highlight the potential efficacy of the assayed by-products on these targets. DPP-IV inhibition ranged from 72.4±2.5% to 31.2±3.4%, showing a moderate to high level of activity, with some by-products having significant inhibition property. For α-amylase, the inhibition range was even higher, being between 93.8±0.35% and 51.9±1.22%. This indicated a strong inhibitory effect of the tested by-products. Finally, samples at the 5 mg/mL concentration did not appear to have inhibition on α-glucosidase. On this basis, a higher concentration was tested (10 mg/mL), and some samples showed a weak inhibitory activity on α-glucosidase (range: 33.86±2.35% - 3.94±0.34%). This suggests that these by-products could be effective in regulating DPP-IV and α-amylase activity and suggest a possible coadjutant effect in the management of postprandial glucose levels. Overall, if confirmed, the inhibition percentages reflect a promising potential for these by-products as functional ingredients useful for people suffering for metabolic diseases. Further studies are still ongoing to determine the IC_{50} of each sample, in order to better understand the dose-dependent response of samples and to optimise concentrations for future experiments and applications.

4. Conclusions

The results of this study confirm the data from our previous studies [3,8] demonstrating that spectrophotometric methods are effective in obtaining preliminary information on the phenolic composition of grape samples, showing a satisfactory correlation with antioxidant activity. In particular, pomace, especially when it contains seeds, was identified as a valuable source of polyphenols with remarkable antioxidant properties. Furthermore, these by-products show promising inhibitory activities of dipeptidyl peptidase IV (DPP-IV), pancreatic α-amylase and α-glucosidase, enzymes that play a critical role in the regulation of postprandial glycemia. These preliminary results suggest that winemaking by-products can be exploited as a rich source of bioactive compounds, suitable for incorporation into dietary supplements or other functional products. This adds not only value to winemaking process, but also highlights the potential of these by-products in contributing to more sustainable practices within the wine industry. By exploiting the bioactive properties of pomace, there is an opportunity to develop innovative products that support health and well-being, while promoting environmental sustainability through an efficient use of agricultural waste. Further research and development in this area could lead to significant advances in both nutraceuticals and sustainable agriculture, highlighting the multiple benefits of using winemaking by-products.

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