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Original article

Monitoring of the use of spent grape pomace after industrial distillation as a potent antioxidant and enzymes inhibitor

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Summary Spent grape pomace (SGP) obtained after the production of *Grappa* has been recognised as a source of antioxidant bioactive compounds. This paper aimed to assess the antioxidant activity and inhibitory effects on enzymes related to non-communicable diseases of white and red SGP, as well as to monitor the impact of storage on these bioactivities. In both pomaces, distillation did not preserve some of the bioactivities; however, after 1 month of storage, there was an improvement. The phenolic profile remained relatively stable during storage while the inhibition of α-glucosidase (AG) was improved. These results confirmed the remaining bioactivity of SGP, demonstrating its potential use in the food industry.

Keywords acetylcholinesterase inhibition, antioxidant activity, *Grappa*, spent grape pomace, α-amylase inhibition, α-glucosidase inhibition.

Introduction

Grape pomaces (GP) boost the production of animal feed, the conversion of grape seed and skin powders in food additives (Spigno *et al.*, 2017; Caponio *et al.*, 2023) and the extraction of grape seed oil (Jin *et al.*, 2021). In addition, GP are collected by distilleries for recovering ethanol and tartaric acid. A traditional product called *Grappa* is made in Italy by distillation of grape pomace (Da Porto & Decorti, 2008; Da Porto *et al.*, 2010).

Depending on the pomace employed, red or white, different types of *Grappa* can be produced (Cisneros-Yupanqui *et al.*, 2021). The distillation of grape pomace for grappa production is of great significance in Italy's industrial sector, reaching a production of 74 200 hL of pure alcohol equivalents in 2021 (Associazione Nazionale Industriali Distillatori di Alcoli ed Acquaviti, 2021).

This traditional strategy of valorisation generates an additional by-product: the spent grape pomace (SGP). Based on a biorefinery approach, the waste discarded by one process should be the input for another process (Bustamante *et al.*, 2008), and, therefore, it could be of interest to think about a better sustainable and economically advantageous exploitation of this grappa by-product. Contrary to fresh pomace, SGP obtained after the distillation has been little studied so far (Bordiga *et al.*, 2015). Previous research has shown the potential of SGP as a source of antioxidants since this bioactivity, especially due to the content of phenolic compounds, did not decrease significantly after the distillation of grape pomace (Cisneros-Yupanqui *et al.*, 2021), fostering its valorisation.

Phenolic compounds have been related to inhibiting key enzymes responsible for several non-communicable diseases, such as α-amylase and α-glucosidase in the case of type-2 diabetes mellitus (T2DM) (Cisneros-Yupanqui *et al.*, 2023) and the enzyme acetylcholinesterase, which activity is associated with the promotion of β-amyloid fibrils formation (Silman & Sussman, 2005) that is related to dementia and Alzheimer's Disease (AD) pathogenesis (Aluko, 2021). Nowadays, commercial drugs such as acarbose and voglibose are used for the control of glycemic response as well as tacrine, and galantamine for inhibiting acetylcholinesterase activity. However, there is a big incidence of several side effects such as abdominal discomfort, diarrhoea and flatulence (Hameed *et al.*, 2022), chronic hepatotoxicity issues (Yang *et al.*, 2023), allergic reactions (rash, rash or itching), trouble with breathing or swallowing, the feeling of tightness in the chest (Kaur *et al.*, 2022). Therefore, the use of other alternatives with similar efficacy and reduced side effects is highly encouraged (Zøotek *et al.*, 2020).

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Based on these considerations, the aims of this paper are to assess the antioxidant activity and inhibitory effects of white and red SGP on enzymes associated with type-2 diabetes mellitus and dementia. Additionally, the influence of storage on these bioactivities will be investigated, considering previous research that highlighted its impact on antioxidant activity. The hypotheses propose that SGP retains antioxidant activity post-distillation, exhibits enhanced enzyme inhibition with storage and that phenolic compounds contribute significantly to these bioactive properties. By addressing these objectives, this research aims to contribute to the understanding of SGP's potential as a source of natural bioactive compounds with implications for functional food development and applications.

Materials and methods

Materials and reagents

The industrial distillery Bonollo Umberto SpA (Padova, Italy) supplied white and red spent grape pomaces (SGPs) derived mainly from Chardonnay and Valpolicella cultivars (*Vitis vinifera*). The following substances were obtained from Sigma-Aldrich (St. Louis, MO, USA): ethanol, Folin–Ciocalteu's phenols reagent, Na_2CO_3, NaOH, HCl, acetic acid, $FeCl_3$, 2,3,5-Triphenyltetrazolium (TPTZ), tetramethylchromane-2-carboxylic acid (Trolox), 4-nitrophenyl-α-D-glucopyranoside, 5,5′-dithiobis (2-nitrobenzoic acid), acetylthiocholine and HPLC standards, including gallic acid, catechin, procyanidin B2 and epicatechin.

Preparation of spent grape pomace (SGP) and extraction procedure

The SGPs, which included seeds, skin and stalks, were subjected to oven drying at 60 °C until a constant weight was achieved over a period of 24 h. The dried materials were then ground into powder form with a water-cooled laboratory mill (IKA Werke M20, Germany) to achieve a particle size smaller than 500 μm. The resulting powders were stored in darkness at 4 °C until further analysis. Moisture content was determined for all SGPs, and all subsequent analytical measurements were expressed on a dry matter basis.

Next, an ethanolic extraction was carried out following a previously described method (Cisneros-Yupanqui *et al.*, 2021). In this process, the samples (at a ratio of 1:10 w/v) were dissolved in a 70% ethanol aqueous solution and heated at 50 °C for 45 min in a water bath with agitation at 140 r.p.m. Following this step, the solid residues were eliminated through centrifugation (9500 ***g*** for 5 min at 4 °C) and filtration. The remaining pellets underwent a second extraction using 5 mL of the same solvent, following the aforementioned steps. The filtrates from both extractions were combined, and triplicate assessments were conducted for total phenolic content (TPC), antioxidant activity (AOA), phenolic profile and inhibitory activity against α-amylase (AM), α-glucosidase (AG) and acetylcholinesterase (AChE) in all the extracts.

Spent grape pomace storage

SGP was assessed at 0, 1, 2 and 3 months of storage at room temperature and all the analytical assays described in the following section were carried out in all the samples.

Analytical assays

Total phenolic content (TPC)

The TPC was evaluated by the Folin-Ciocalteau method (Campos *et al.*, 2022). In summary, a diluted sample of 500 μL was combined with 250 μL of 1 N Folin-Ciocalteau's reagent and 1250 μL of 7.5% $NaCO_3$. A blank solution was prepared with water instead of a sample. After incubating in darkness for 30 min, the absorbance value was measured at 755 nm (Varian Carry 50 Bio UV/Vis spectrophotometer). The results were expressed as mg of gallic acid equivalent per g of dried matter (mg GAE/g).

Antioxidant activity (AOA)

The AOA was assessed through the ferric-reducing antioxidant potential (FRAP) spectrophotometric assay (Solari-Godiño *et al.*, 2017). The FRAP reagent was prepared by mixing 2.5 mL of 0.01 M of TPTZ in HCl 40 mM, 2.5 mL of an aqueous solution of 0.02 M $FeCl_3$ and 25 mL of 0.2 M of sodium acetate buffer (0.2 M Sodium acetate/0.2 M acetic acid). A FRAP volume of 900 μL was mixed with 100 μL of the diluted sample and incubated at 37 °C for 30 min. A blank solution was prepared with ethanol. The absorbance was measured at 593 nm using a Varian Carry 50 Bio UV/Vis spectrophotometer. The results were expressed in mg of Trolox equivalent per g of dried matter (mg TE/g).

Quantification of phenolic compounds by HPLC

The phenolic profile analysis was conducted using an HPLC system (Agilent 1200 series, Palo Alto, USA) equipped with a diode array detector. Prior to injection into the column, the samples underwent filtration using a 0.22 μm cellulose acetate filter (Millipore, Bedford, USA). These following phenolic compounds were identified: gallic acid, catechin, epicatechin, procyanidin B1 and procyanidin B2, based on their retention time, using the Poroshell column 120 EC-C18 (3.0 × 100 mm) with a particle size of 2.7 μm, along with a Zorbax precolumn. The operational parameters were as follows:

Solvent A consisted of 95% (0.1 M H_3PO_4), while solvent B contained 5% (absolute methanol with 0.5% 0.1 M H_3PO_4). The elution process utilised a gradient starting from 5% solvent B and progressing to 80% solvent B. The column temperature was maintained at 35 °C, and a sample injection volume of 5 μL was used. The flow rate was set at 0.4 mL/min, with detection performed at a wavelength of 280 nm. The total running time for the analysis was 35 min. The calibration curve ($r^2 > 0.99$) was carried out, according to a previous work (Cisneros-Yupanqui *et al.*, 2021).

α-Amylase (AM)-inhibitory assay

To achieve a final concentration of 1 U/mL α-amylase, each extract was combined with an enzyme solution in a 1:1 ratio (v/v). The mixture was then allowed to incubate for 15 min at a temperature of 23 °C. The remaining activity of α-amylase was determined following the procedure outlined in the Sigma–Aldrich method (Sigma-Aldrich Enzymatic Assay of α-Amylase (EC 3.2.1.1), 1955). The absorbance was measured at 540 nm, the negative control being the enzyme without inhibitors. The inhibition rate of α-amylase was calculated using the following formula:

$$\text{Inhibition}\ (\%) = 100 - \left(A_{540\text{Blank corrected sample}} / A_{540\text{Blank corrected control}}\right) \times 100$$

The results are expressed based on the extract concentration (mg/mL) that inhibited 50% of the enzyme activity (IC_{50}).

α-Glucosidase (AG)-inhibitory assay

The reaction mixture contained 10 μL of extract (several extract concentrations were tested to determine the IC_{50}) and 30 μL of α-glucosidase (0.13 U/mL, G5003-100UN, Sigma-Aldrich, Merck, Darmstadt, Germany). This mixture was placed in a microplate reader (SPECTROstar Nano Microplate Reader, BMG LABTECH, Ortenberg, Germany) and incubated for 15 min at 37 °C. Then, 25 μL of 1 mM 4-nitrophenyl-α-D-glucopyranoside was introduced. The reaction mixture was then shaken and further incubated at 37 °C for 10 min. To terminate the reaction, 60 μL of 0.2 M Na_2CO_3 solution was added. Blank samples were prepared by adding the extract after the reaction was terminated. The absorbance at 405 nm was measured using a microplate reader. A negative control was established using the enzyme without any inhibitor. The α-glucosidase inhibition percentage was calculated from the blank-corrected data using the following formula (Saifuddin & Raziah, 2008):

$$\%\text{Inhibition} = 100 - \left(A_{405\text{Blank corrected sample}} / A_{405\text{Blank corrected control}}\right) \times 100$$

The results are expressed based on the IC_{50}.

Acetylcholinesterase (AChE)-inhibitory assay

The *in vitro* AChE-inhibitory assay was conducted under experimental conditions based on a previously described method (Lobbens *et al.*, 2017) with slight modifications. The assay was carried out in a 96-well microplate. In each well of the microplate, there were 30 μL of AChE (with a final concentration of 0.05 U/mL, C3389-500 U, Sigma–Aldrich, Merck, Darmstadt, Germany), 125 μL of 1.5 mM 5,5′-dithiobis (2-nitrobenzoic acid) diluted in phosphate-buffered saline (PBS) at pH 7.5, 45 μL of PBS at pH 7.5, and 25 μL of either the test solution or the negative control (water). To create a blank sample, buffer replaced the enzyme. The microplate was briefly shaken for 10 s and then incubated at 30 °C for 5 min. Subsequently, 30 μL of 7.5 mM dissolved in water was added to each well. The absorbance at 412 nm was measured every 30 s for a duration of 1 min. The obtained data were adjusted by subtracting the blank values and plotted against time to calculate the reaction rate (slope of the plot). The calculation of inhibition involved comparing the reaction rate observed in the test solution to that of the negative control. To ensure accuracy, the entire experiment was repeated three times. The inhibition was then expressed as a percentage using the following formula:

$$\%\text{Inhibition} = 100 - \left(\text{Slope}_{\text{sample}} / \text{Slope}_{\text{negative control}}\right) \times 100$$

The results are expressed based on the IC_{50}.

Statistical analysis

The results presented are expressed as the mean ± standard deviation obtained from three independent experiments ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by the *post-hoc* Duncan test ($P < 0.05$) to assess significant differences. Correlations were evaluated using the Pearson coefficient in the Statgraphics Centurion XIX software (StatPoint Inc., Rockville, MD, USA).

Results and discussion

Total phenolic content (TPC) and phenolic profile of SGP along the storage

In both types of grape pomace, there was an increase in the TPC after the distillation, from 34.72 ± 3.40 mg GA/g to 38.72 ± 0.96 mg GA/g and from 31.30 ± 4.74 mg GA/g to 32.05 ± 0.56 mg GA/g for white and red pomace, respectively. This data are in agreement with a previous research where white SGP obtained a higher content of polyphenols (Cisneros-Yupanqui *et al.*, 2021). Figure 1 shows the effect of

3 months of storage in the TPC, both pomaces presenting the same trend of not changing significantly ($P < 0.05$), in comparison to the starting point. However, it is suggested a storage of 1 month, where it is reached the maximum TPC of both SGP, 44.94 ± 0.40 mg GA/g and 38.19 ± 0.97 mg GA/g for white and red, respectively. This increase may be attributed to the potential presence of residual activity in certain endogenous enzymes within the samples, such as pectin methyl esterase (Zocca *et al.*, 2007), which has been reported to exhibit significant thermal stability and demonstrate an augmentation in activity during storage (Song *et al.*, 2023).

On the contrary, the phenolic profile of both types of pomaces is similar (Table 1), the white one presenting slightly better values for all of them, but not for procyanidin B2. This difference is explained by the specific vinification procedures employed in each case (Pascual *et al.*, 2022). Regarding white pomace, it is noticeable the significantly higher content of gallic acid (3.44 ± 0.03 mg/g) than in red pomace (1.62 ± 0.05 mg/g), as previously found for white pomace from winemaking (Jara-Palacios *et al.*, 2015). However, distillation decreased both values significantly ($P < 0.05$), as reported previously (Cisneros-Yupanqui *et al.*, 2021). This process also had a considerable impact on the content of procyanidin B2 in red pomace (from 3.28 ± 0.01 mg/g to 2.50 ± 0.01 mg/g) and procyanidin B1 in both pomaces. In this last case, the values decreased from 2.96 ± 0.04 mg/g to 1.40 ± 0.02 mg/g, and from 2.04 ± 0.07 mg/g to 1.48 ± 0.01 mg/g for white and red pomace, respectively. Nevertheless, as reported for the TPC, there was a significant ($P < 0.05$) increase in the content of catechin (white pomace) and epicatechin (red pomace) after distillation, showing a high correlation [$r = 0.8489$ ($P < 0.05$)] among these variables (TPC and catechins). These results confirmed the impact of distillation on the phenolic content, which has been reported in several research (de Elguea-Culebras *et al.*, 2022).

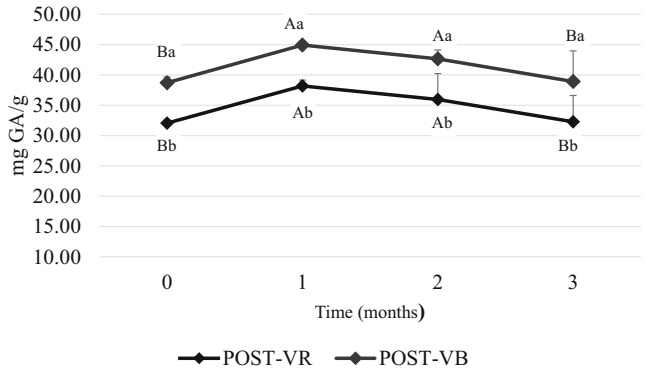

Figure 1 Monitoring the total phenolic content (mg GAE/g) of white and red SGP along the storage time. Data are expressed as the mean ± standard deviation ($n = 3$). Different letters in the figure indicate statistically significant differences ($P < 0.05$) due to the time of storage (capital letters) and type of sample (lowercase letters), according to ANOVA (two-way) and the Duncan test. POST-VR, spent red pomace; POST-VB, spent white pomace.

The impact of the storage on the phenolic profile is shown in Table 2. After 1 month, there was a significant decrease ($P < 0.05$) in the content of almost all the phenolic compounds assessed. This reduction could be attributed to the involvement of certain phenols in the Maillard reaction and their ability to trap dicarbonyls (Han *et al.*, 2022). However, there was a slight, but significant ($P < 0.05$) increase in the content of gallic acid and procyanidin B1 in white and red SGP, respectively. Regardless of the variations within the storage, the phenolic profile did not show a considerable change, suggesting the stability of these bioactive compounds present in both SGPs over 3 months.

Antioxidant activity (AOA) of SGP along the storage

Similar to the TPC, the antioxidant activity (AOA) of white pomace was higher than the red one, these two variables being positively correlated [$r = 0.6283$ ($P < 0.05$)]. However, this low value is due to the

Table 1 Phenolic profile of red and white grape pomace

	White pomace		Red pomace	
Polyphenols (mg/g)	**PRE-VB**	**White SPG**	**PRE-VR**	**Red SPG**
Catechin	4.33 ± 0.04^{b}	4.81 ± 0.04^{a}	3.82 ± 0.01^{c}	4.06 ± 0.02^{c}
Epicatechin	3.84 ± 0.01^{a}	3.62 ± 0.04^{c}	3.64 ± 0.02^{c}	3.74 ± 0.02^{b}
Gallic acid	3.44 ± 0.03^{a}	2.72 ± 0.09^{b}	1.62 ± 0.05^{c}	1.44 ± 0.03^{d}
Procyanidin B1	2.96 ± 0.04^{a}	1.40 ± 0.02^{d}	2.04 ± 0.07^{b}	1.48 ± 0.01^{c}
Procyanidin B2	2.76 ± 0.01^{b}	2.78 ± 0.01^{b}	3.28 ± 0.01^{a}	2.50 ± 0.01^{b}

Data are expressed as the mean ± standard deviation ($n = 3$). Different letters in the same row indicate statistically significant differences ($P < 0.05$), according to ANOVA (one-way) and the Duncan test. PRE-VB, white pomace before distillation; PRE-VR, red pomace before distillation; SPG, spent grape pomace.

Table 2 Monitoring the phenolic profile of spent grape pomace (mg/g) along the storage time

Phenolic compound (mg/g)	Red SPG				White SPG			
	$t = 0$	$t = 1$	$t = 2$	$t = 3$	$t = 0$	$t = 1$	$t = 2$	$t = 3$
Catechin	4.06 ± 0.02^{Ab}	3.67 ± 0.03^{Bb}	3.83 ± 0.01^{Ab}	3.84 ± 0.02^{Bb}	4.81 ± 0.04^{Aa}	4.64 ± 0.04^{Ba}	5.24 ± 0.01^{Aa}	4.62 ± 0.01^{Ba}
Epicatechin	3.74 ± 0.02^{Aa}	3.45 ± 0.04^{Ba}	3.45 ± 0.04^{Ca}	3.43 ± 0.03^{Ba}	3.62 ± 0.04^{Ab}	3.44 ± 0.03^{Bb}	3.18 ± 0.01^{Cb}	3.42 ± 0.01^{Bb}
Gallic acid	1.44 ± 0.03^{Bb}	1.35 ± 0.04^{Ab}	1.30 ± 0.09^{Bb}	1.34 ± 0.02^{Bb}	2.72 ± 0.09^{Ba}	3.18 ± 0.05^{Aa}	2.56 ± 0.04^{Ba}	2.63 ± 0.03^{Ba}
Procyanidin B1	1.48 ± 0.00^{Ba}	1.79 ± 0.04^{Aa}	1.62 ± 0.01^{ABa}	1.66 ± 0.04^{ABa}	1.40 ± 0.02^{Bb}	1.35 ± 0.02^{Ab}	1.42 ± 0.01^{ABb}	1.35 ± 0.04^{ABb}
Procyanidin B2	2.50 ± 0.01^{Aa}	2.32 ± 0.02^{Ba}	2.23 ± 0.02^{Aa}	2.25 ± 0.01^{Aa}	2.78 ± 0.01^{Aa}	1.54 ± 0.05^{Ba}	2.78 ± 0.02^{Aa}	2.65 ± 0.01^{Aa}

Data are expressed as the mean ± standard deviation ($n = 3$). Different capital letters in the same row indicate statistically significant differences ($P < 0.05$) due to the time of storage while different lowercase letters in the same column indicate differences on the type of sample, according to ANOVA (two-way) and the Duncan test. SPG, spent grape pomace.

significant decrease of the AOA after distillation, on the contrary to the TPC. The reduction in the AOA was especially noticed in red pomace, where the value almost dropped to half (from 65.93 ± 8.61 mg TE/g to 35.87 ± 3.81 mg TE/g). The decrease in the AOA could be related to the heat generated during the distillation, which has been shown to affect this bioactivity (de Lima Marsiglia *et al.*, 2023). Despite of not being highly correlated with the TPC, AOA and gallic acid present a Pearson coefficient of 93.34%, similar to the one found in a by-product from the winery (Vitas *et al.*, 2023). This fact shows the importance of combining Folin-Ciocalteau, which is a preliminary trial, with other assays.

Figure 2 shows the effect of 3 months of storage in the AOA, both pomaces presenting a slight, but significant decrease ($P < 0.05$), in comparison to the starting point. However, there is a considerable increase after 1 month in both cases. Along the 3 months of storage, the AOA of white SGP is more than twice higher than red SGP. This pattern exhibits a correlation with the total phenolic content (TPC) at a level of 53.60% ($P < 0.05$), suggesting the presence of molecules other than polyphenols that possess AOA. Among these compounds, certain Maillard reaction products (MRP) were identified to exhibit AOA (Vhangani & Van Wyk, 2016). These MRP could be generated as a result of the heat treatment of grape pomaces during distillation. They could exert antioxidant effects along the storage, where their content was reported to increase (Li *et al.*, 2022). The polymerisation of some compounds may be another responsible for this bioactivity as well as the antioxidant fibre present in these SGPs (Yang *et al.*, 2022).

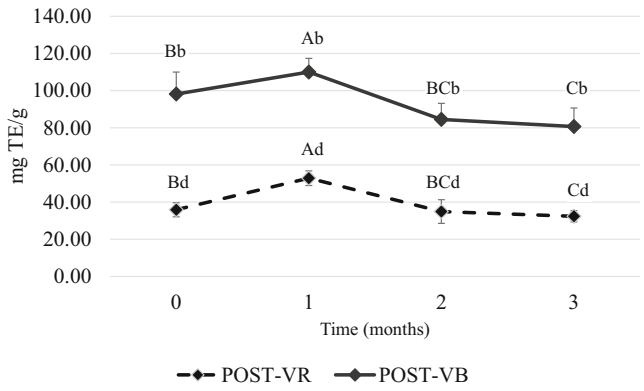

Figure 2 Monitoring the antioxidant activity (μmol TE/g) of white and red SGP along the storage time. Data are expressed as the mean ± standard deviation ($n = 3$). Different letters in the figure indicate statistically significant differences ($P < 0.05$) due to the time of storage (capital letters) and type of sample (lowercase letters), according to ANOVA (two-way) and the Duncan test. POST-VR, spent red pomace; POST-VB, spent white pomace.

Inhibitory potential of spent grape pomace (SGP) towards α-Amylase (AM), α- glucosidase (AG) and acetylcholinesterase (AChE) along the storage

The inhibition of the AM activity was only found in white grape pomace, reaching an initial IC_{50} value of 56.45 ± 0.05 mg/mL. Phenolic compounds were reported to be responsible for inhibiting AM in different food sources (Sardabi *et al.*, 2022). Catechins, whose content was higher in white than in red SPG, present a Pearson correlation of 98.98% with this bioactivity. This compound has a high affinity to AM as well as binds other sites than the active one (Miao *et al.*, 2014). Gallic acid [$r = 0.9877$ ($P < 0.05$)], epicatechin [$r = 0.9776$ ($P < 0.05$)] and, especially, procyanidin B1 [$r = 0.9996$ ($P < 0.05$)] were other phenolic compounds with a high correlation with the inhibition of AM. The significantly lower content of epicatechin in white than in red SGP could be attributed to its polymerisation into more intricate molecules, such as procyanidin B1. These complex molecules possess a greater ability to form crosslinks with different compounds than the individual monomeric phenolic compounds (Lavelli *et al.*, 2016). Despite the fact white SPG had a smaller amount of procyanidin B1, these ones may present a higher degree

Table 3 Monitoring the α-amylase activity, in terms of the IC_{50} value (mg/mL) of white grape pomace along the storage time

	Amylase (IC_{50}, mg/mL)			
Sample	***t* = 0**	***t* = 1**	***t* = 2**	***t* = 3**
White SPG	100.67 ± 0.07^{b}	74.76 ± 0.05^{d}	132.80 ± 0.08^{a}	78.06 ± 0.06^{c}

Data are expressed as the mean ± standard deviation (n = 3). Different letters in the figure indicate statistically significant differences ($P < 0.05$) due to the time of storage, according to ANOVA (one-way) and the Duncan test.

of polymerisation, as found in other white varieties (Tkacz *et al.*, 2019). However, distillation did not favour the SGP capacity of inhibiting AM since the IC_{50} increased to 100.67 ± 0.07 mg/mL. The reduction in the content of procyanidins caused by the high temperatures reached in distillation could be the responsible for this loss in the bioactivity (Khanal *et al.*, 2010).

The behaviour of the AM inhibition activity in related to the storage time (Xu *et al.*, 2019), as shown in Table 3. The best inhibition was found after 1 month (IC_{50} of 74.76 ± 0.05 mg/mL). This behaviour is correlated with the content of gallic acid [$r = 0.7856$ ($P < 0.05$)] and epicatechin [$r = 0.7612$ ($P < 0.05$)], maybe due to the polymerisation of these compounds along the time (Yang *et al.*, 2022). In addition, a correlation of 58.43% ($P < 0.05$) was found with the AOA, suggesting the presence of other molecules than phenolic compounds involved in this bioactivity.

Similarly, distillation decreased the AG inhibitory activity. In this case, the white SGP obtained a better value (IC_{50} of 154.46 ± 0.82 μg/mL) than the red one (IC_{50} of 308.53 ± 0.95 μg/mL. However, the storage seemed to balance this difference between both SGPs (Fig. 3). Red SGP almost reached the half of the IC_{50} value after 3 months (152.93 ± 1.20 μg/mL) while the white one enhanced its IC_{50} value, too, but not significantly ($P > 0.05$), reaching a final value of 140.29 ± 1.23 μg/mL. The presence of some phenolic compounds such as gallic acid could be responsible for this bioactivity (Oboh *et al.*, 2016) because there was a correlation of 72.48% ($P < 0.05$), and its quantity was higher in white SPG. Moreover, other antioxidants detected in the FRAP assay could be implied in the AG inhibitory activity since a correlation of 73.91% ($P < 0.05$) was found. For example, some MRP, especially the ones derived from fructose– and glucose–tyrosine, have been previously related to this bioactivity (Hwang *et al.*, 2011).

Regarding the inhibition of AChE, white pomace presents a better potential than red pomace with an IC_{50} value of 4.87 ± 0.08 mg/mL and 9.66 ± 0.07 mg/mL, respectively. These results are similar to the ones found previously in red pomace, around 9 mg/mL (Mollica *et al.*, 2021). However, distillation decreased more than three times the AChE inhibition, reaching a

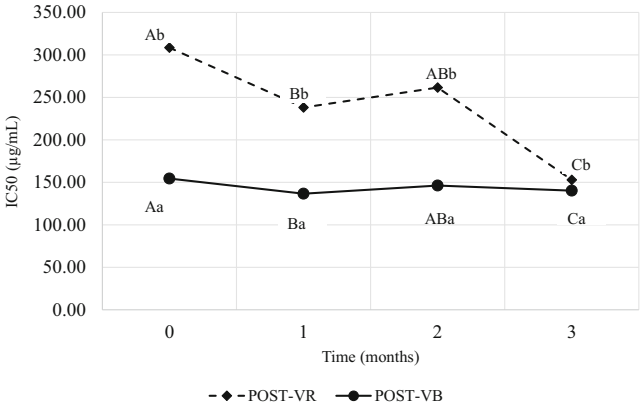

Figure 3 Monitoring the α-glucosidase inhibitory activity, in terms of the IC_{50} value (μg/mL) of white and red SGP along the storage time. Data are expressed as the mean ± standard deviation (n = 3). Different letters in the figure indicate statistically significant differences ($P < 0.05$) due to the time of storage (capital letters) and type of sample (lowercase letters), according to ANOVA (two-way) and the Duncan test. POST-VR, spent red pomace; POST-VB, spent white pomace.

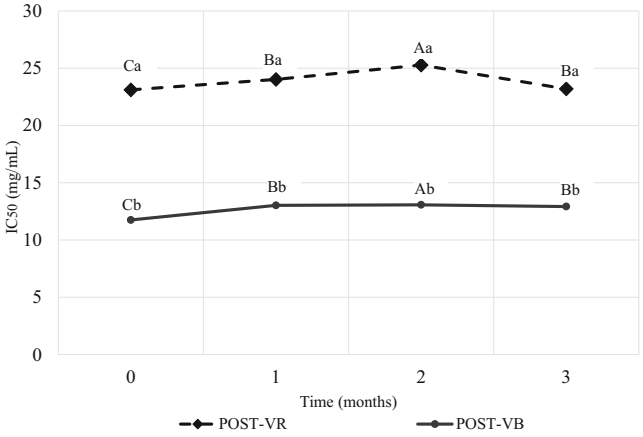

Figure 4 Monitoring the acetylcholinesterase (AChE) activity , in terms of the IC_{50} value (mg/mL) of white and red SGP along the storage time. Data are expressed as the mean ± standard deviation (n = 3). Different letters in the figure indicate statistically significant differences ($P < 0.05$) due to the time of storage (capital letters) and type of sample (lowercase letters), according to ANOVA (two-way) and the Duncan test. POST-VR, spent red pomace; POST-VB, spent white pomace.

final IC_{50} value of 11.75 ± 0.06 mg/mL and 23.11 ± 0.05 mg/mL for white and red pomace, respectively. This behaviour was highly and significantly ($P < 0.05$) correlated with the content of gallic acid (Kade & Rocha, 2013), procyanidin B1 (Xu *et al.*, 2009) and the AOA (Simeonova *et al.*, 2021), presenting a Pearson coefficient of 73.84%, 74.86% and 84.62%, respectively. The storage of both SGPs did not affect the AChE-inhibitory capacity (Fig. 4). The behaviour of both pomaces towards the enzymes studied could be related to the change in the type of inhibition performed. This may be explained by the impact of distillation on the structure of the different molecules present (Jiang *et al.*, 2021), possibly modifying the affinity with the enzymes studied.

Conclusions

In conclusion, the results of this study demonstrated that both white and red SGP experienced an increase in TPC after distillation. However, this operation significantly decreased the AOA, the levels of gallic acid, procyanidins and the inhibition of the enzymes studied. The inhibition of AG and AChE activities was found in both pomaces while AM was only in the white one. The storage of 1 month helped to partially recover almost all the bioactivities lost after distillation. The results highlighted the importance of considering together the effects of distillation and storage of SGP on the bioactive properties of grape pomace.

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Author contributions

Miluska Cisneros-Yupanqui: Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Corrado Rizzi:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Dasha Mihaylova:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Ivelina Deseva:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal). **Anna Lante:** Conceptualization (equal); writing – original draft (equal); writing – review and editing (equal).

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Data availability statement

Data available on request from the authors

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