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ENHANCING OF BIOAVAILABILITY OF VALUABLE COMPOUNDS FROM GRAPE POMACE BY ENZYMATIC TREATMENT

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Abstract

Grape pomace, the most abundant winemaking by-product, represents a possible source of valuable biocompounds that can be valorised in different domains of industry also providing environmental benefits. The investigation performed provides relevant data on the potential of various enzyme mixtures (β-glucanase, hemicellulases, xilanases, pectinases, proteases) to release monosaccharides, amino acids and polyphenols by grape pomace's hydrolysis, thereby increasing its nutritional and functional properties. The reducing sugars, the aminoacids and the polyphenols obtained were determined, and the antioxidant activity was measured before and after the enzymatic treatments of the grape pomace. The researches performed on two varieties of grape pomace indicated that the white variety was noted with a higher quantity of total soluble sugars, while red grape pomace was found to have higher amounts of amino acids, polyphenols and higher antioxidant activity. The enzymatic treatment of grape pomace enhanced the extraction yield by up to 59% for reducing sugars, respectively 55% for polyphenols.

Key words: *antioxidant activity; enzymatic hydrolysis; glucanases; pectinases; polyphenols.*

INTRODUCTION

Grape (*Vitis* spp.), one of the most appreciated fruits, represents a significant crop cultivated all over around the world. Approximately 75% of the entire grape production is utilized for wine-making (Zhu et al., 2015), producing millions of tons of residues, such as grape pomace, which represents 20 to 25% of the total processed grapes. Incineration or storage of grape pomace in landfills results in environmental pollution, and waste management can become a significant problem for wine producers. Efforts are currently being made to valorise grape pomace in different domains of industry as an environmentally sustainable alternative.

Grape pomace mainly consists of grape seeds, skins and stalks. According to Nerantzis and Tataridis (2006) one ton of grape pomace is composed of around 430 kg of grape skins, 250 kg of grape stalks and 230 kg of grape seeds. Seeds are rich in antioxidant compounds, such as phenolic acids, flavonoids and procyanidins. In addition, grape seeds were found to have a high content of oil (between 15 to 18%) which is rich in essential fatty acids, non-digestible carbohydrates, proteins and bioactive compounds such as tocopherols and β-carotene (Fiori et al., 2014). Grape skin and pulp are rich sources of fibers and phenolic compounds depending on the vinification techniques and grape variety considered.

There are numerous studies regarding the use of grape pomace in human nutrition as a source of dietary fiber in bread and biscuits (Mildner-Szkudlarz et al., 2013) or to improve the nutrition profile of frankfurters (Ozvural & Vural, 2011), yoghurt and salad dressing (Tseng & Zhao, 2013). There have also been developed several experimental feeding strategies regarding the use of grape pomace for ruminants (Ianni & Martino, 2020) but also for pigs (Bertol et al., 2017) or poultry (Kumanda et al., 2019).

Beside this, grape pomaces constitute an inexpensive source for the extraction of bioactive compounds that can be used in the pharmaceutical and cosmetic industries (Fontana et al., 2013) due to their potential benefits on health promotion and prevention. However, their efficacy in products may be reduced due to the tight binding to cellulose matrices. Certain mechanisms such as hydrophobic interactions and hydrogen bonds between cell wall polysaccharides and various polyphenols (Lin et al., 2016) were studied in grapes and other plants, therefore the treatment with enzymes (pectinases, cellulases, glucanases) has been performed to release the polyphenols from their matrices in order to enhance their bioavailability and efficacy (Holland et al., 2020).

There are numerous researches investigating the capitalization of grape pomace by extracting bioactive compounds that can be used in the pharmaceutical, cosmetic and food industries.

As enzymes differ in hydrolysis efficacy, the present study was focused on testing some commercial enzymatic complexes based on cellulases, pectinases, glucanases and proteases activity in order to increase the nutritional and functional properties of this by-product through hydrolysis. Enzymatic treatment of white and red grape pomace was performed using commercial enzymatic complexes in different concentrations. The reducing sugar, amino acids and polyphenols obtained were determined, and the antioxidant activity was also measured before and after the enzymatic treatments.

Using grape pomace as valuable source of essential nutrients represents not only a method of valorisation of waste from the wine industry, but also an ecological alternative for their disposal, helping to improve sustainable agricultural practices.

MATERIALS AND METHODS

Substrate

The by-product used in our study was grape pomace from *Vitis vinifera* L., white and red varieties, provided by a Romanian vineyard. To be used in the analysis performed, the grape pomace was ground to increase the accessibility of enzymes to the complex polysaccharides (starch, celluloses, hemicelluloses).

Enzymes hydrolysis

Enzymatic hydrolysis was performed using three commercial enzyme complexes representing combinations of xilanases and β-glucanase (1), pectinases and hemicellulases (2) and proteases (3), that were added to the substrate as a mixture. Concentrations (E.C.) between 0.25% and 1% of the enzyme mixture relative to the reaction medium were tested. The enzymatic treatment was performed for 20 h, at 50°C, pH = 5 to 5.5, on a stirred water bath at a constant stirring rate of 120 rpm. Control samples without enzymes added were considered for all conditions.

Total soluble sugars determination

The determination of total soluble sugars was performed according to the Somogyi-Nelson method described by Iordachescu & Dumitru (1988). First non-reducing sugars were converted into reducing sugars by hydrolysis with hydrochloric acid. The reducing sugars obtained react with alkaline copper tartrate when heated, forming cuprous oxide. This reduces arsenomolybdic acid and forms molybdenum blue. The measurements of absorbance were achieved with a UV/Visible spectrophotometer (ThermoSpectronic Helios) at 510 nm. The results were expressed in g% FW (fresh weight).

Total polyphenols determination

The total polyphenols content was determined according to the modified Folin-Ciocalteu assay (Singleton et al., 1999). The method is based on chemical reduction of Folin-Ciocalteu reagent (a mixture of tungsten and molybdenum oxides) and measuring the intensity of the obtained blue colour at 750 nm. The polyphenols content values were expressed in terms of gallic acid equivalent, which is a common reference compound (mg GAE/100 g FW).

Free amino acids determination

The determination of the amino acids content was performed using the ninhydrin assay (Suna et al., 2006), a spectrophotometric method based on the reaction between amino acids and ninhydrin, resulting in the development of a purple-coloured compound known as Ruhemann's purple. The colour intensity of the formed complex is proportional to the concentration of amino acids in the solution, so absorbance was measured at 570 nm. The results were expressed in g% FW.

DPPH scavenging assay

Total antioxidant capacity (radical scavenging activity) was determined using the stable free radical DPPH (diphenylpicryl-hydrazyl) method according to the procedure adapted by Brand-Williams et al. (1995) for complex matrices. 2 ml of 100 µM solution of DPPH in methanol were mixed with 1 ml of different concentrations of grape pomace extract in 80% aqueous methanol. The absorbance (A) of the reaction mixture was measured at 515 nm after 30 min incubation in dark at room temperature. The percentage of the radical scavenging activity (RSA) was calculated according to the formula:

$$\% \text{ RSA} = (1-[A_{sample}/A_{control\ t=0}])100^{-1}$$

DPPH solution in 80% methanol was used as a control. The IC_{50} parameter (mg/ml) for each sample, defined as the concentration of sample which is required to scavenge 50% of DPPH free radicals, was calculated from the linear regression curve of the sample extracts against the percentage of the radical scavenging activity.

Statistical analyses

All measurements were carried out in triplicate, and the results are presented as means ± standard deviation (SD). The significance of the influence of the enzymatic treatment on the measured parameters was assessed by one-way ANOVA test for means discrimination at a confidence level of 95% ($P < 0.05$) using Microsoft Excel Office 2019 for Windows.

RESULTS AND DISCUSSIONS

The chemical composition of grape pomace can vary depending on factors such as grape variety, obtaining method, climate or location. High variation in grape pomace composition was found considering that fermentation of the whole grape mass is required for making red wine, while the rose and white wines are made by juice fermentation, therefore multiple ways of grape pomace capitalization are possible (Antonic et al., 2020).

Three commercial enzymatic complexes containing carbohydrases and proteases were tested in this study with the aim of enhancing the yield of chemical compounds extraction from grape pomace. The hydrolysis degree of complex sugars, the release of amino-acids and polyphenols and the antioxidant capacity of grape by-products after enzymatic reaction with carbohydrases and proteases for 20 h were evaluated.

Influence of hydrolysis treatment on the total soluble sugar content

An enzymatic mixture containing a combination of carbohydrases (complex 1, containing xilanases and β-glucanase; complex 2, containing pectinases and hemicellululases) in a 1:1 ratio was tested on white and red grape pomaces. The total sugars content was determined in grape pomaces samples after treatment with concentration varying between 0.25% and 1% of the enzymatic combination compared to control samples (with no enzyme added).

The analysis performed on the control samples indicated a lower reducing sugars content in red grape pomace (0.20 ± 0.01 g%) compare to white grape pomace (2.82 ± 0.06 g%) (Figure 1). Recent studies have found that the content of the simple sugars in red grape pomaces is usually low due to the difference in red and white wine production technology. Jin et al., in the year 2019, found that the total sugar content was in the range of 7.79 to 14.3 g/kg DW for red grape pomace, while higher amounts of soluble sugars were present in white grape pomace (69.8 g/kg, 75.2 g/kg, and 107.6 g/kg DW for Petit Manseng, Viognier, and Vidal Blanc pomace, respectively).

White grape pomace is obtained by pressing grapes for juice, while red grape pomace is collected after the fermentation of grape pulps for several days. During this process, most sugars are consumed by yeast cells (Jin et al., 2019; Antonic et al., 2020).

The high amounts of sugars remaining in white grape pomace can be recovered and used in various fields, such as for the production of aldonic acids, used in the cosmetics and plastic industry (Tomaszewska et al., 2018) or for fermentation to produce biochemicals or biofuels (Corbin et al., 2015).

Adding a combination of carbohydrases to grape pomace, degrading the cell wall polysaccharides, increased the content of reducing sugars.

The results obtained in this study revealed that the enzymatic treatment was more effective on the red grape pomace, resulting in about 59% increase of the amount of reducing sugars content compared to the control samples (with no enzymatic treatment) (Figure 1). In the case of white grape pomace, the 0.5% concentration of the enzyme complex led to obtaining the highest amount of released sugars, but the increase in reducing sugar content after enzymatic treatment was lower (26%) compared to the red pomace.

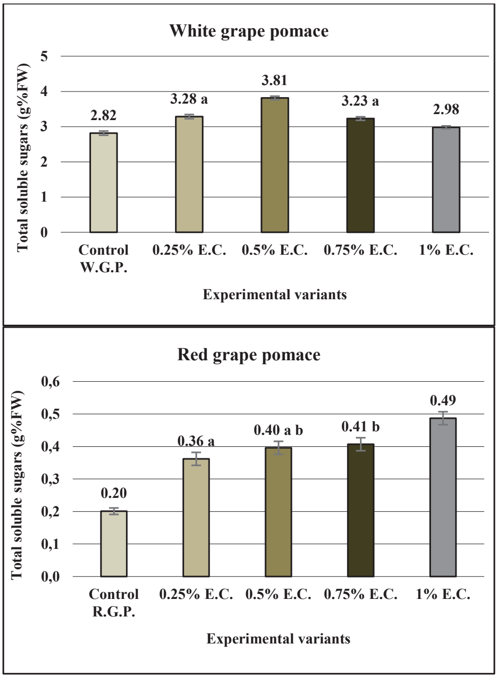

Figure 1. Total soluble sugars content in the grape pomace samples
Data expressed as means ± standard deviation of three samples analyzed separately; bars marked with the same letters are not significantly different ($P < 0.05$)

These results provide data about the potential of some commercial enzymatic complexes on the release of reducing sugars from grape pomace, improving their nutritional value, in accordance with the results found in other studies (Chamorro et al., 2012; Alberici et al., 2020). For example, Chamorro et al. (2012) found that the addition of carbohydrases to grape pomace, either alone or in combination, degraded the cell wall polysaccharides, increasing the content of monosaccharides.

Influence of hydrolysis treatment on the amino acids content

Enzymatic complexes containing proteases were tested on white and red grape pomace. The amino acids content was determined in grape pomace samples after enzymatic treatment with concentration varying between 0.25% and 1% of enzymatic complex compared with control samples (with no enzyme added).

In general, there is more information available about the protein content of grape pomace than about the amino acid content in this by-product. Thus, the crude protein content reported ranged from 6.3 g% to 10.6 g% in white grape pomace, while the same parameter ranged from 11.5 g% to 13.0 g% in red grape pomace (Jin et al., 2019; Marin et al., 2020). However, some authors found that in dry grape marc with seeds or without seeds have similar amounts of essential amino acids as those found in cereals: for instance, lysine ranges 0.544 g% in the seed-less grape marc, higher than the values reported for corn (0.261 to 0.399 g%) and wheat (0.352 to 0.395 g%) (Olteanu et al., 2014).

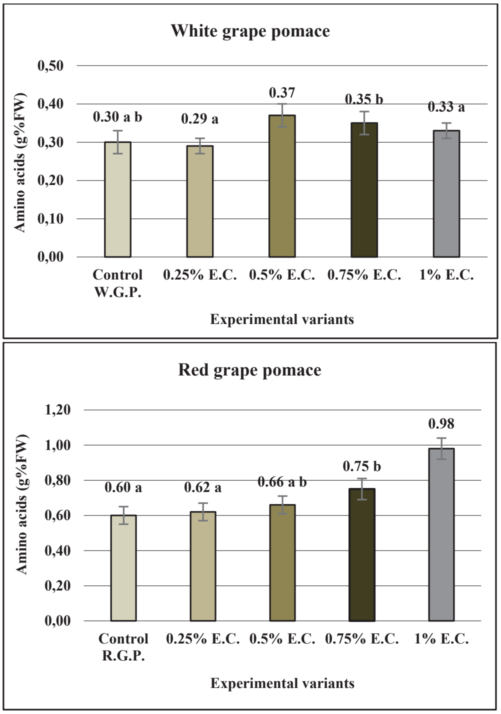

Figure 2. Amino acids content in the grape pomace samples
Data expressed as means ± standard deviation of three samples analyzed separately; bars marked with the same letters are not significantly different ($P < 0.05$)

The research performed in this study (Figure 2) found a higher content in free amino acids in red grape pomace (0.6 ± 0.05 g%) compare to that from white pomace (0.3 ± 0.03 g%) in the control samples. The enzymatic treatments resulted in a low increase of the amino acids content in the white pomace, with around 19% more amino acids determined in the sample with the highest enzymes concentration added compare to the control. In contrast, the red pomace samples had 39% more amino acids after enzymatic treatment compared to the control.

Influence of hydrolysis treatment on the total polyphenols content

In recent years grape pomace is frequently used as a source of polyphenols. Because of an incomplete extraction during the winemaking process, grape pomace contains high amounts of phenolic compounds, which are known as secondary plant metabolites with potential beneficial effects on human health due to their antioxidant activity.

In this study, the same enzymatic mixture containing a combination of carbohydrases as described for the sugar determination was tested on white and red grape pomaces regarding polyphenols content. The total polyphenols content was determined in grape pomace samples after treatment with concentration varying between 0.25% and 1% of enzymatic mixture compared with control samples (with no enzyme added).

The grape pomace varieties tested showed high but diverse contents of polyphenols. Red pomace had 1.8 times more polyphenols (365.21 ± 3.40 mg GAE/100 g FW) than white pomace (203.42 ± 8.53 mg GAE/100 g FW) before the enzymatic treatment (Figure 3). Also, Jin et al. (2019) found differences between white and red grape pomace after a comparative evaluation of polyphenols content, which ranged from 11.8 to 32.1 g/kg DW gallic acid equivalents in white grape pomace and from 10.4 to 64.8 g/kg DW gallic acid equivalents in red grape pomace.

The amounts of polyphenols in the extracts after enzymatic treatment showed an important increase of 55% in the case of white pomace and less significant in the case of red pomace (increase of 13%). The effect of enzymatic concentrations used in the experiment was found to be dependent on the grape variety. White grape pomace was found to be more sensitive to enzymatic action with the highest polyphenols yield (446.65 ± 17.13 mg GAE/100 g FW) obtained when white pomace was treated with the maximum concentration (1%) of enzymatic mixture.

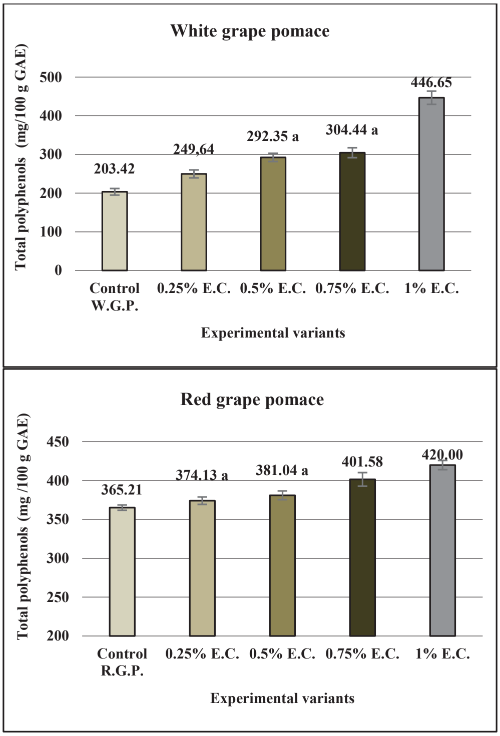

Figure 3. Total polyphenols content in the grape pomace samples

Data expressed as means ± standard deviation of three samples analyzed separately; bars marked with the same letters are not significantly different ($P < 0.05$)

Starting from the hypothesis that enzyme degradation of plant cell wall polysaccharides can potentially enhance the release of bioactive phenolics, also Chamorro et al., in the year 2012, and Meini et al., in the year 2019, found that pectinases and cellulases were effective in releasing polyphenolics bound to the cellulose matrices of grape skins and seeds. According to Meini et al., the enzymatic treatment enhanced the extraction yield of phenolics by up to 66% and its antioxidant capacity by up to 80%. Thus, they found that tannase increases the antioxidant capacity of the extract by releasing of gallic acid, while cellulase favours the releasing of malvidin-3-O-glucoside and p-coumaric acid.

Influence of hydrolysis treatment on the antioxidant capacity

Antioxidant capacity (radical scavenging activity) of grape pomace varieties was evaluated in the same samples used for polyphenols determination and the IC_{50} values (the concentration of sample that is required to scavenge 50% of DPPH free radicals) were calculated for further comparison (Table 1).

The measurements performed before the enzymatic treatment indicated that the highest antioxidant activity was found in red pomace (4.65 mg/ml expressed as IC_{50} value). The lowest scavenging capacity was recorded in the white pomace, which required therefore a higher concentration (11.43 mg/ml) to scavenge 50% of DPPH free radicals compared to red pomace.

After enzymatic treatment, the DPPH radical scavenging activity was found to be higher in the grape pomace samples compared to untreated grape pomace. The increase in antioxidant activity was more pronounced in the white grape variety (2.21 times higher) than in the red variety, where enzymatic treatment determined an increase of only 1.35 times of the antioxidant activity in relation to the control sample.

Table 1. IC_{50} values of DPPH scavenging activities in the grape pomace samples

Grape pomace variety	Experimental variants	IC_{50} (mg/ml)
White	Control (0% E.C.*)	11.43
	0.25% E.C.	7.00
	0.5% E.C.	5.77
	0.75% E.C.	5.17
	1% E.C.	6.97
Red	Control (0% E.C.)	4.65
	0.25% E.C.	4.15
	0.5% E.C.	3.44
	0.75% E.C.	3.76
	1% E.C.	4.05

*E.C. = enzymatic concentration in the reaction mixture

Antioxidant activity shows the same tendency as the phenols content, most likely due to the fact that increasing amounts of phenols results in a higher free radical scavenging. The relationship between the IC_{50} values and total polyphenols contents was performed via Pearson's correlation coefficient (R). The variables polyphenols content and IC_{50} measured at the tested enzymatic concentration showed a good correlation for red grape pomace (R = 0.9580) (Figure 4), but a weaker correlation was calculated for the white grape pomace (R = 0.7798).

Also, Chamorro et al., in the year 2012, found that enzymatic treatment with pectinase facilitated the release of gallic acid from grape pomace increasing the antioxidant activity.

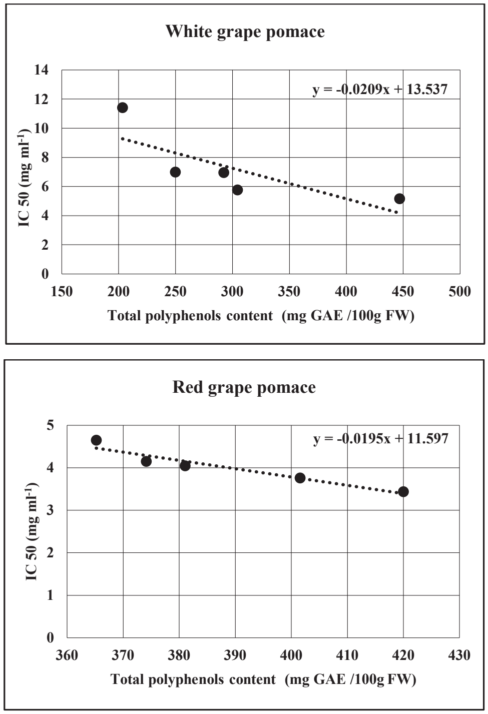

Figure 4. Correlation between total polyphenols content and antioxidant activity

The valuable content in bioactive compounds of grape pomace can have multiple applications. Further research indicated that due to its high antioxidant activity, grape pomace polyphenolic extract can be used as a replacement of synthetic antioxidants in food products in order to obtain the extension of its shelf-life (Dodan et al., 2021).

CONCLUSIONS

Grape pomaces, the most abundant winemaking by-product, represent a potential source of valuable biocompounds which can be recovered and used in various fields. The comparative study of the tested grape pomace varieties indicated that the white variety was noted with a higher content of total soluble sugars, while red grape pomace was found to have a higher content of amino acids, polyphenols and higher antioxidant activity.

The research performed also provided relevant data about the potential of different enzymes combination (xilanases, β-glucanase, pectinases, hemicellulases, proteases) to release monosaccharides, amino acids and polyphenols from grape pomace, which could improve the nutritional value and the antioxidant capacity of the by-product.

Reusing grape pomace as a nutritional supplement both for human and animals or as functional ingredients in the pharmaceutical and cosmetic industries would provide a sustainable alternative for recovering and valorizing the wine industry by-product, while also promoting environmental advantages.

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