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Scientific editors

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Chapter 15

Industrial use of bioenergy and high-value molecules extracted from winemaking by-products. Ecological aspects

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Abstract

From the concept of circularity, a new industrial revolution is arising where waste as unwanted or unusable material is virtually unknown. In present times, indicators describe the main constraints posed on our world`s future development: population growth, carbon dioxide in the atmosphere, energy consumption, overuse of minerals and natural resources, and shortage of water, which leads to a future zero-waste society. In this context, a study was developed to explore the potential applications of winemaking sub-products, known as grape pomace (GP), which have incomplete exploitation and part of which is dumped as biowaste. In European countries, the winemaking industry converts 22 million tonnes annually of grapes into wine, giving rise to 5 million tonnes of GP as a product. The goal of this research was to examine the antioxidant capabilities of GP polyphenols in order to identify useful industrial uses. With a total phenolic content (TPC) of 86 mg GAE/g DM in Loureiro variety seed and only 35.1 mg GAE/g DM in Vinhão variety, with very significant differences ($p < 0.01$), GP can offer a wide range of products with high-value bioactive molecules. In the skin fraction, the TPC value was higher in Vinhão variety than Loureiro variety (120 and

24 mg GAE/g DM, respectively). The findings of TPC and the antioxidant activity as determined by DPPH activity are correlated, with r = 0.988 for Vinhão and r = 0.975 for Loureiro. From the seeds of the grape types Loureiro and Vinhão, a high-yield edible oil content of 16.5% and 17.7%, respectively, was found. When compared to other biomass heating values, the heating value results of GP samples after the fat and polyphenol extraction, which are between 16.9 and 17.2 MJ/kg, show that GP extracted residue has the potential for energy recovery. This byproduct offers fresh prospects for alternative industrial uses when taking into account the quantities of GP that are produced each year and its chemical characteristics.

Keywords: grape pomace; polyphenol; thermal energy; high heating value

1. Introduction

Sustainability of natural resources in order to maintain the earth environmental system in equilibrium is a demanding action to the humankind present survival. In this context, the wine production industry was the target of this study, in particular the sub-products after the wine making. The aim was to study the chemical composition of grape pomace and to evaluate the best utility for this sub-product, studying the potential use of this biomass for producing high value molecules and energy.

A broader use of renewable resources and the substitution of fossil based materials and products by renewable raw materials, like agriculture by-products will be an important and necessary step. This solution could help to reduce the present environmental problems, since the renewable material is a source to feed other industry raw material needs. The European Commission in its Lead Market Initiative (EC, 2007) identified bio-based products, made from renewable raw materials such as plants and trees, as one of the promising future markets. This is a closed cycle of transformation of matter with full appliance.

Grape is the world's largest fruit crop with an annual production of more than 67 million tons. Grapes and products obtained from them, such as wine, grape juice, jams, and raisins, have then an obvious economic importance. Eighty percent of the worldwide grape production is used in winemaking (Fontana Ariel R., 2013). The wine industry produces millions of tons of by-products that are named grape pomace, GP, which mainly consists of stalks, skins, and seeds and represents a waste management issue both environmentally and economically. Economically because most wineries in Portugal are small enterprises, scattered in the territory, which do not have the tools and the ability to properly manage and dispose them. So, from the ecological point of view, a common practice for

GP is to lay down in the fields, in order to be composted by aerobic decomposition. During this biodegradation process, GP remains exposed and is a source of contamination, mainly because of the volume and many natural products that it contains. The organic load has significant antimicrobial and phytotoxic activity, which significantly limits the action of microorganisms involved in the process of biodegradation. As a result, the process of biodegradation is slowed down. Another problem is the high levels of phenolic compounds that inhibit seed germination. Regarding GP use in livestock feed, some animals show intolerance to certain components, such as condensed tannins and polymeric polyphenols (lignin), which negatively affect digestibility because they inhibit cellulolytic and proteolytic enzymes as well as the growth of rumen bacteria (Fontana Ariel R., 2013).

With the increase of consumers' awareness for the use of additives in foods and the attention that functional foods have acquired in recent years, there is a need for the identification of alternative natural and safer sources of food additives, namely antioxidants, colorants and preserving additives, as well as natural functional foods. Adding to this, modern industries are focused on diminishing the environmental impact of industrial byproducts. Therefore, most attention has been paid to the recovery of bioactive phenolic compounds from grape byproducts from the winemaking industry. Portugal is the fifth largest wine producer in the EU-28 (Table 15.1) with a production estimated at 5,886 khL in 2014.

Table 15.1. Wine production* trend in the EU-28 (khL)

	2011/12	2012/13	2013/14	Rank
Italy	43,072	40,057	44,900	1
Spain	33,397	31,123	44,600	2
France	50,890	40,609	44,100	3
Germany	9,258	9,000	8,500	4
Portugal	**5,609**	**6,140**	**6,740**	**5**
Romania	4,700	4,100	5,400	6
Greece	2,750	3,150	3,700	7
Hungary	2,822	2,243	2,450	8
Austria	2,814	2,155	2,252	9
Other EU-28 countries	3,214	2,558	4,911	10
EU-28	158,527	141,135	167,553	11

* Volume of product removed from fermenters after the first natural fermentation of the must of fresh grapes (juices and other musts were excluded).

Source: (GAIN-FAS, 2014).

This study was developed with samples from GP of the wine production with appellation of origin "Vinho Verde". This is obtained from a demarcated region of grape wine cultivated in the northwest of Portugal (Figure 15.1).

Polyphenols are the most important phytochemicals in grapes because they possess many biological activities and health-promoting benefits to the plants. Grape pomace is characterized by high-phenolic contents because of poor extraction during winemaking. The bioactive substances are used worthwhile and thus support sustainable agricultural production (Kammerer Dietmar, 2004). Although, the absolute amount as well as the relative proportion of these beneficial compounds in GP is conditioned by a myriad of factors including genetic load of the separate grape varieties, agro-climatic conditions, fertilization procedures, and soil properties, among others. On the other hand, the specific wine-making processes as well and the time between the generation of by-products and valorization activities, as well as the characteristics of the recycling and recover procedures have a direct impact on the final concentration of phenolic compounds in the GP and, therefore, on the potential use as a source of bioactive phytochemicals.

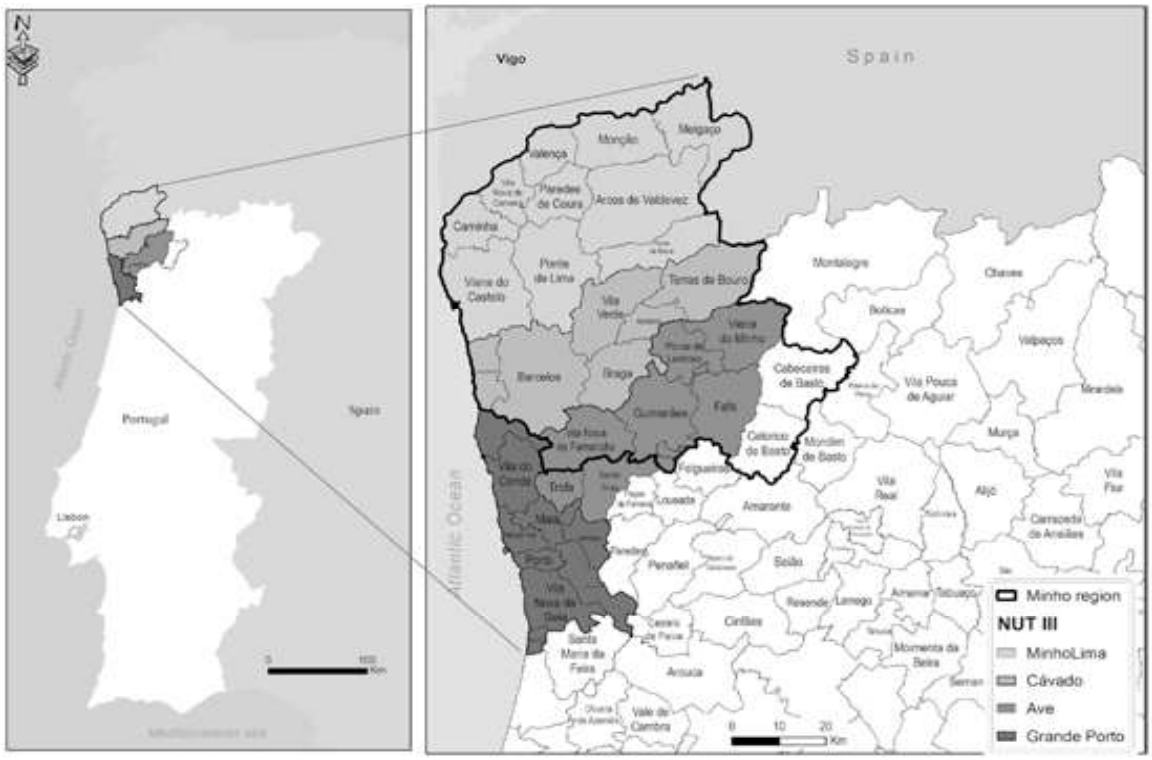

Figure 15.1. Map representing the demarcated region of "Vinho Verde"
Source: (IGP, 2010).

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The objective of this study was to evaluate the potential application of grape pomace, from Vinhão and Loureiro grape varieties, as bioenergy or as raw material in other industrial processes. To achieve this, two specific goals were established: determine the GP bioactive content, through the assays of total phenolic content (TPC) and antioxidant activity (DPPH); Evaluate the high heating value (HHV) in GP and extracted GP as a form of bioenergy.

2. Experimental development

The study for the by-products recovery after the winemaking process with Loureiro and Vinhão grape varieties was an innovative theme. A general characterization of the grape pomace, including the extraction and quantification of the bioactive compounds and finally the determination of the calorific value of grape pomace and extracted samples was investigated. In the present chapter, reagents, standards and solutions used throughout all the experiments are described. Furthermore, aspects regarding the sample preparation and latter analysis are also explained.

2.1. General experimental conditions

Analytical grade reagents were purchased from different suppliers and used for all experiments. For the preparation of all solutions, water from Barnstead™ E-Pure™ Ultrapure Water Purification Systems was used throughout the work. All the standards and the samples were weighted in a Mettler Toledo analytical

balance (model AE 200) with the accuracy of decimal of miligram. Standard stock solutions were prepared by rigorous weighing the respective reagent, followed by dissolution in the appropriate solvent (water, buffer solution, methanol, or mixture of the previous solvents). All reagents that appear in the experimental procedure, without the reference grade, were pa grade.

2.2. Sampling and transportation

Samples of grape pomace resulting from winemaking with the species *Vitis Vinifera L.* of the varieties Loureiro and Vinhão cultivated and harvested in the region of Lima, Alto Minho, Portugal were provided by Adega Cooperativa de Ponte da Barca, in September. The GP from the white Loureiro was collected immediately after separation from the must in the step of decantation, before fermentation as a mixture (skin and seed) from big opened air tanks shown in Figure 15.2. The GP from the red grapes Vinhão was collected after the fermentation of the must, as a mixture of skin and seed. The stalks from both varieties were collected in the back of the factory, Figure 15.3, after the stalking step. Many authors consider GP consisting of a mixture of all the fractions, stalk, skin and seed, while others consider only the skin and the seed. In this work, GP means the stalks, skin and seed and GP mixture refers to the skin and seed. The sampling for both varieties was done during three different periods of time in September, collecting about 3 kg of GP in each sampling visit, making a total of 9 kg of each variety. All the samples were transported in fully and closed vessels at room temperature for about one hour to the laboratory and then followed the pretreatment.

Figure 15.2. Storage of grape pomace in the factory

Figure 15.3. Storage of stalks in the factory

2.3. Pretreatment and codification of samples

In the lab, fresh grape pomace was divided. Half amount was dried at 30 °C in a drying oven, Heraeus Model UT 6060 for four days. After, it was vacuum packed and stored until analysis. Before analyses, the seeds and skins from each sampling visit were manually separated and the same fraction from the three visits was mixed together and dried at 103°C. The other half amount of each variety was vacuum packed and it was stored at –20°C, after manual separation of the skin and the seed of white Loureiro. The analyses of the heating value were determined on samples dried at 103 °C. The samples stored at –20 °C were lyophilized in a Christ Alpha 1-2 LD Freeze Dryer with a Rotary vane pump RZ 2.5 and used for the studies on polyphenol extraction. For all the analyses the samples were ground in a Taurus blender (Aromatic Model, Type: SP- 7407) until homogenous flour to approximately 8-meshes. Samples were codified by B and T standing for white ("Branco") and red ("Tinto") GP, respectively, followed by L for Loureiro and V for Vinhão varieties. Finally the letters E, P and G stands for stalks ("Engaço"), skin ("Película") and seed ("Grainha") respectively. Samples of GP with a fourth letter, E, in the beginning stands for extracted samples (Table 15.2).

Table 15.2. Codification of samples from Adega Cooperativa de Ponte da Barca

CODE	PART OF GRAPE	COLOUR	VARIETY
BLE	Stalk	White	Loureiro
BLM	Mixture*		
BLP	Skin		
BLG	Seed		
TVE	Stalk	Red	Vinhão
TVM	Mixture		
TVP	Skin		
TVG	Seed		
Samples after extraction			
EBLE	Extracted Stalk	White	Loureiro
EBLM	Extracted Mixture		
ETVE	Extracted Stalk	Red	Vinhão
ETVM	Extracted Mixture		

* The mixture contains the seed and the skin of the grapes.

2.4. Polyphenols extraction

The extractions were made according to author Shoeb (Shoeb Mohammad, 2005). A Soxhlet extractor JP SELECTA with thimble for Soxhlet extractor, filter paper (QL 100, 185mm Fisher Scientific), n-hexane 95% (J.T. Baker, analytical reagent standards), dichloromethane (J.T. Baker analytical reagent standards) and methanol (Riedel-deHaën, Analytical Reagent and J.T Baker analytical reagent standards, were used.

Lyophilized grape pomace sample, about 20 g, was placed inside a thimble made from thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a 250 mL glass flask with hexane as the first extraction solvent (220 mL). Dichloromethane and methanol also 220 mL each were used as the second and third extraction solvents by this order. The Soxhlet was then equipped with a condenser. Each solvent was heated to reflux for 6h at the temperature of 60, 40 and 60°C, respectively. After extraction, the solvent was removed, in a rotary evaporator, yielding the extracted compounds. After weighting (m1) the solid residue was re-dissolved immediately in methanol and stored at –20°C until analyses. After the three solvent extractions

the non-soluble portion of the extracted solid remaining in the thimble, was collected for high heating value determination.

$$\%\ \text{yield} = \frac{m2_{\text{flask (g)}} - m1_{\text{flask (g)}}}{m_{\text{sample (g)}}} \times 100$$

$m1_{\text{flask}}$ is the initial weight of empty flask in g
$m2_{\text{flask}}$ is the weight of the flask after each solvent extraction (hexane, dichloromethane, methanol) in g
m_{sample} is the weight of dry sample

2.5. Total phenolic content – Folin & Ciocalteu

The total phenolic content in GP was determined by the Folin-Ciocalteu colorimetric method. (Lafka Theodora-Ioanna, 2007). The colorimetric total phenolic assay with Folin & Ciocalteu reagent relies on the transfer of electrons, in alkaline medium, from phenolic compounds to phosphomolybdic/ phosphotungstic acid complexes, which act as oxidant. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 765 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols. The exact chemical composition of the reagent is not known, but the reaction that occurs is the reduction from Mo (VI) to Mo (V) by transfer of one electron. A double-beam ultraviolet– visible spectrophotometer, Hitachi U-3210, Folin & Ciocalteu reagent, 2.0M (Sigma Aldrich), saturated solution of Na_2CO_3 (anhydrous, J.T.BAKER), , Gallic acid monohydrate (Sigma Aldrich), methanol (Riedel-deHaën, analytical reagent) were used.

Methanolic GP extract (m2 in 50 mL) was appropriately diluted (Table 15.3) and 0.5 mL of this solution was transferred to a volumetric flask of 25 mL and it was added 20 mL deionized water and 0.625 mL of Folin–Ciocalteu reagent. After mixing, it was left standing for 3 min and then 2.5 mL of saturated solution of Na_2CO_3 was added. The content was mixed again and diluted to volume with deionized water. After two hours, the absorbance of the sample was measured against a blank by using a spectrophotometer. Gallic acid served as the standard for preparing the calibration curve with the concentrations of 10; 25; 50; 75; 100; 125; 150; 175; 200; 250 and 300 mg/L, following the same procedure as the samples (0.5 mL of standard solutions and blank in a volumetric flask of 25 mL).

Table 15.3. Dilution factor for the TPC determination

Sample	BLE	BLM	BLP	BLG	TVE	TVM	TVP	TVG
d.f	2	20	10	20	4	20	2	2

From concentration (mg/L) formula:

$$C\ (mg/L) = \left[\frac{Abs_{sample} - b}{m}\right] \times d.f$$

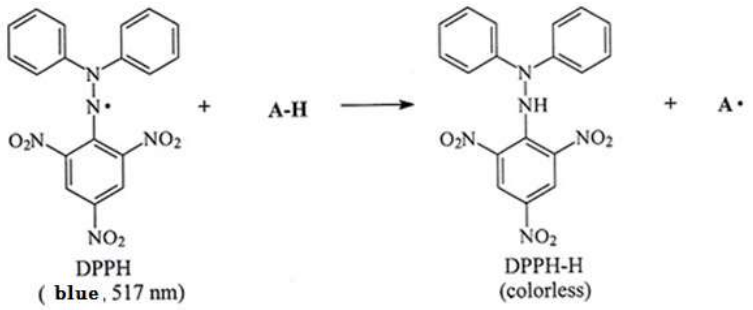
the following formula was used to obtain the results in mg gallic acid equivalent/g DM:

$$mg\ GAE/gDM = \frac{C\ (mg/L) \times V(L) \times (\frac{m_1(g)}{m_2(g)})}{m_{sample}(g)}$$

Abs is the absorbance obtained from the spectrophotometer,
m is the slope of the line
C is the concentration in mg/L,
b is the y-intercept of the line,
d.f is the dilution factor
V is the volume in L, for the m_2 dissolution (0.05 L)
m_1 is the mass of the extraction, in g
m_2 is the mass of the sampling, in g
m_{sample} is the mass of the GP lyophilized sample in g, used in the extraction

2.6. Antioxidant activity – DPPH assay

The free radical scavenging activity of the GP fractions was measured in vitro by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described below (Qian Deng, 2011). The method is based on the molecular absorption of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The solution of this radical, which has a blue color, is measured spectrophotometrically at 517nm. When this solution is added to a substance with antioxidant activity (A-H), then the DPPH radical is reduced with the recruitment of a hydrogen atom or an electron and is converted to 1,1-diphenyl-2-picrylhydrazyl which is colorless. This reaction takes place in the dark during 30 min and gives a yellow color, depending on the extension of the reaction of DPPH reduction in the presence of an antioxidant (AH):



The molecular absorption was measured in a double-beam ultraviolet–visible spectrophotometer Hitachi U-3210, using DPPH solution: 9 mg of DPPH dissolved in 100 mL methanol and then diluted 30/50 (DPPH, Sigma-Aldrich); (+) L-Ascorbic acid (Panreac) and methanol (Riedel-deHaën, analytical reagent).

Methanolic GP extract (m2 in 50 mL) was appropriately diluted (Table 15.4) and 0.5 mL of this solution was transferred in a cuvette and mixed thoroughly with 1.5 mL of DPPH–methanol reagent and allowed to stand at room temperature for 30 min prior to measure the solution absorbance at 517 nm. Ascorbic acid was used as standard for preparing the calibration curve with concentrations 1.00; 2.50; 5.00; 7.50; 10.00; 20.00; 30.00; 35.00 and 40.00 mg/L followed the same procedure as the samples.

Table 15.4. Dilution factor for the DPPH determination

Sample	BLE	BLM	BLP	BLG	TVE	TVM	TVP	TVG
d.f	5	100	10	100	20	20	2	10

From the equation of the concentration (mg/L):

$$C\ (mg/L) = \left[\frac{Abs_{sample} - b}{m}\right] \times d.f$$

was introduced in the following formula to obtain the results in mg Ascorbic Acid equivalent/gDM:

$$mg\ AAE/g\,DM = \frac{C\ (mg/L) \times V(L) \times \left(\frac{m_1(g)}{m_2(g)}\right)}{m_{sample}(g)}$$

Abs is the absorbance obtained from the spectrophotometer,
m is the slope of the line

C is the concentration in mg/L,
B is the y-intercept of the line,
d.f is the dilution factor
V is the volume in L, for the m2 dissolution (0.05 L)
m_1 is the mass of the extraction, in g
m_2 is the mass of the sampling, in g
m_{sample} is the mass of the GP lyophilized sample in g, used in the extraction

2.7. High heating value determination

Heat released in a chemical reaction can be determined experimentally by using an adiabatic bomb calorimeter, as the case of heat of combustion. The reaction must proceed without any side reactions and sufficiently fast, so the heat exchange with the surroundings is negligible. In such calorimeter, the combustion reaction occurs in a closed container under constant volume ("bomb"). The bomb is immersed in a weighted quantity of water and surrounded by an adiabatic shield that serves as a heat insulator. Continuous bath stirring ensures that heat is distributed evenly in the calorimeter. The bomb and the water bath, which are in direct thermal contact, constitute an adiabatic bomb calorimeter and the increase in the water temperature is used to calculate the heat of the reaction. The equipment was a Parr Calorimeter 6772, a press for pellet production, Parr with a furnace line function Heraeus, and wire (Parr), benzoic acid (one gram pellets standardized for bomb calorimeter, Parr).

For the procedure for the HHV determination in grape pomace sample, about 0.5 to 1.0 g, was placed in the pellet press to prepare the pellet. The final pellet was weighted accurately. Carefully, it was placed in the sample cup with tweezers. It was measured approximately 10 cm of ignition wire and attached to the electrodes (Figure 15.4A) and each cap was slide downward to complete the connection. The sample cup (with the sample sitting in the center) was placed in the cup holder and the wire bended in a V-shape so that it almost touches the surface of the pellet (about 1 mm separation) and do not touch the cup surface. Figure 15.4B illustrates the proper installation and sample placement. Deionized water (1.0 mL) was pipetted into the bomb to absorb the oxides of nitrogen and sulfur formed from nitrogen and sulfur present in air and sample.

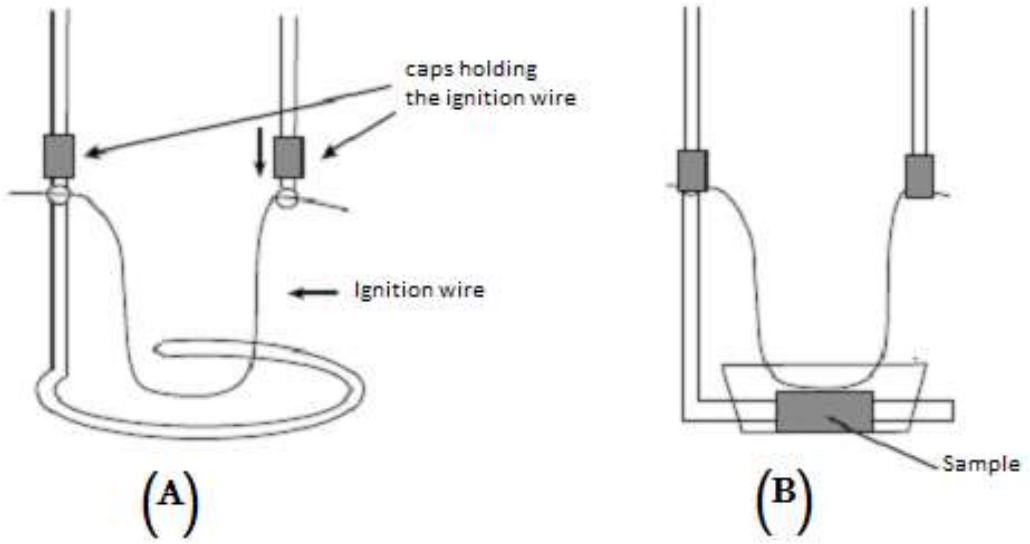


Figure 15.4. (A) Attachment of the ignition wire. (B) Schematic of the sample support stand

Care must be taken not to disturb the sample when sealing and charging the bomb. The head assembly slides into the bomb cylinder, followed by screw opening the vent cap on the head assembly to allow air to be expelled, and the head pushed down and after the vent cap was tightly closed. Later on, the oxygen connection was installed by secure the bomb in the bench clamp and slipped on the oxygen tank connection hose to the pin on the head assembly. The oxygen tank valve was opened to fill the bomb slowly by watching the gauge in the oxygen bottle as the bomb pressure was rising to the desired filling pressure (30 atm). Once this pressure was reached, the control valve and then the tank valve were closed. The filling of the bomb should not be very fast because it can blow the sample out of the sample cup.

After the bucket was filled with 2,000 ± 0.5 g of water it was checked to see that it is resting properly in the jacket, confirming the four pegs on the bottom of the jacket, which hold the bucket in place. Carefully the charged bomb was placed in the bucket, noting that it rests on the raised circular area on the bottom of the bucket and making sure that there are no oxygen leaks, by watching if there are bubbles. The ignition wires were connected to the terminal socket on the bomb head, avoiding touching with the fingers the water. The cover on the jacket was placed and the screw attached to the lid fitted into the screw hole in the ledge of the jacket. Finally the stirrer was turned on by hand to be sure that it runs freely, and then the drive belt slipped onto the pulley. In some samples it is also needed

the spike weight. If a sample does not ignite easily or press well, then the spiking method of ignition can be used to promote complete combustion of the sample. The heat produced by the combustion aid must be subtracted from the total energy release. In this work a known amount of benzoic acid was added with the sample as a medium to compress easily the sample and make pellet. The benzoic acid is removed from the calculation of the heating value. When the calorimeter was finished any unburned fuse wire length still attached to the electrodes was measured and subtracted from 10 cm, in the calculation for the heating value. Also after the end of each analysis the cup was heated in a muffle furnace, maintained at 550 ^{o}C till a constant weight to weight the unburned sample. Ten independent measurements of the benzoic acid standard were carried out and six independent measurements of each GP samples.

For calculation, the heat produced by the reaction $q_{rxn} = -(q_{water} + q_{bomb})$can be accurately determined by measuring the temperature increase in the known amount of surrounding water. This result obtained automatic from the equipment.

2.8. Statistical analyses

In this study three types of statistical analysis were carried out. For the comparison between the same fraction from the two varieties (e.g. BLE versus TVE) a t-test was used after the evaluation of homogeneity variances of the two samples. A paired t-test was used for the comparison for a lot of pairs, between the same fraction from the two varieties when the results were very different between one pair to another. Finally, the comparison of averages of three or more samples (e.g. BLE versus BLG versus BLP) in the same variety, was made using ANOVA followed by the Tukey's post hoc test. Significant differences were defined for a $p < 0.05$ and very significant differences were defined for a $p < 0.01$. The calculations were made with XLSTAT 2014.4.10 add-in (Addinsoft, Paris, France) for Microsoft Excel 2010 (Microsoft, Redmond, United States of America).

3. Results and Discussion

Considering the different studies on grape pomace in the literature, this study is innovative about these varieties, Loureiro and Vinhão. Two varieties of GP, composed of stalks, skins and seeds collected from Adega as fresh samples were studied, however the mixture (seed and skin) was also studied because the main results presented in the literature are about the mixture. The analyses of TPC and

DPPH were done in duplicate, while the analysis of HHV was done in six replicates. Results were expressed as mean ± standard deviation ($\bar{x} \pm sd$). For the total phenolic content, antioxidant activity and HHV, the statistical comparison was applied between the same fractions from both varieties.

3.1. Polyphenols quantification in GP

3.1.1. Results of the extraction process

The phenolic derivatives distributed in plant tissues are classified by their structures. Thus, the soluble polyphenols with a low molecular weight are located in the vacuoles of the cells, while the insoluble phenolic derivatives (e.g. polymeric lignins) constitute the structural components of the cell walls contributing to the stability and morphology of the plants (Santos-Buelga Celestino, 2012). The process of extraction releases phenolic derivatives from cells and tissues, leading to qualitative and quantitative determination. With high water content it is recommended the refrigeration before the process of extraction, to inactivate the enzyme functions and protect the unstable polyphenols. Furthermore, the extraction of polyphenols is facilitated when preceded by refrigeration at low temperatures because the crystals of the water are formed and destroyed the cellular walls favoring the release of intracellular material. Finally, another practice followed is the lyophilization of the samples, which takes place at low temperatures and leaves intact the polyphenols, allowing the conservation of the samples for a sufficiently long time followed by the use of a homogenizer to rupture the cell tissue and reduce the size particle. Subsequently, the immersion in an appropriate solvent is carried out, to extract the phenolics derivatives by diffusion. The extraction of polyphenols from plant material can be affected by a number of factors, such as the chemical composition, the process used for the extraction, the extraction conditions, the storage time, the tissue size and the presence of undesirable substances and the sample treatment for extraction. The extracts are mixtures of various polyphenols which are soluble in the solvent however they contain also molecules that are not phenols, such as fats, terpenes, and chlorophylls. To remove the latter, often it requires fractionation based on the polarity of the substances (Santos-Buelga Celestino, 2012). The solubility of the polyphenols is dependent on the polarity of solvent used, the polymerization degree and the interaction with other molecules (e.g., proteins) to form insoluble products. For these reasons there is not a single procedure to extract all the phenolic derivatives from a plant sample. The most common technique applied

is the extraction, depends on the author, as it could be the solid-liquid extraction, the Soxhlet extraction, the supercritical fluid extraction etc. Some authors for the fractionation of the undesirable substances, use hexane to remove the fat, dichloromethane to remove the chlorophylls and other pigments and only after that, polar protic media such as hydro alcoholic solutions, to extract the polyphenols. Methanol exhibits the highest capacity to extract phenolics even though the ethanol is cheaper and safer and should be preferable in the case of later food applications. The published results are not conclusive about an ideal solvent, and different mixtures have been proposed (Fontana Ariel R., 2013).

Table 15.5. Extraction yields obtained from Loureiro and Vinhão lyophilised GP samples

Type of Sample	Hexane (%)	Dichloromethane (%)	Methanol (%)
BLE	1.09 ± 0.08	0.40 ± 0.01	34 ± 3.1
BLM	10.9 ± 0.14	1.49 ± 0.14	18.1 ± 0.62
BLP	4.7 ± 0.34	3.36 ± 0.11	40 ± 4.1
BLG	16.5 ± 0.12	0.43 ± 0.01	18.6 ± 0.20
TVE	1.8 ± 0.22	0.44 ± 0.03	16.5 ± 0.11
TVM	13.15 ± 0.03	1.09 ± 0.02	11 ± 1.1
TVP	4.6 ± 0.21	1.67 ± 0.12	28 ± 1.2
TVG	16.7 ± 0.18	0.60 ± 0.06	7.4 ± 0.39

In the present study the GPE was obtained by using firstly hexane, followed by dichloromethane and finally methanol was selected in order to obtain a high yield of polyphenols. The total extraction yields obtained from Loureiro and Vinhão GP are presented in Table 15.5. The yields from hexane showed a higher percentage in the seeds of the two varieties with 16.5% and 16.7% from Loureiro and Vinhão respectively. The dichloromethane extraction showed higher yield in the skin with a percentage of 3.36% in Loureiro and 1.67% in Vinhão. The high extraction yield with methanol, which contains the polyphenols, could be explained because of the presence of other substances, than polyphenols, which were not determined. The highest yield obtained, was from the skin (40 %) and the stalk (34 %) from white Loureiro GP. The extraction yields with methanol from another study were Chardonnay 23.3%, Macabeu 32.4%, Parellada 17.2% and Premsal Blanc 33.3% (González-Centeno M.R, 2013). Authors have published the results of TPC and antioxidant activity and do not present the yield, so the comparison cannot be so extensive.

3.1.2. Results of total phenolic content (TPC) and antioxidant activity (DPPH)

The method of Folin & Ciocalteu (FC), for measuring the total phenolics is a photometric method which was originally developed in 1927 for determining proteins, taking into advantage the fact that the reagent used, reacted with the phenol ring of tyrosine, forming a colored product. Thereafter Singleton and Rossi (Singleton V. L., 1965) improved the method and used it for determination of total phenols in the wine. The process became very popular and widely used since then to determine the phenolic content of various natural products. Although the FC process is used to determine the phenolic substances, actually it is used to determine the reducing ability of the sample, since the reaction carried out in this process is a redox reaction. Therefore, the above method can be considered as a method of measuring antioxidant capacity. The mechanism of this reaction belongs to the category of transport electron, so it should not be surprising that the "polyphenol profile" determined by this method shows good linear correlation with antioxidant capacity determined by other antioxidant methods which also transfer electrons, for example like the ferric reducing ability of plasma (FRAP) or 1,1-diphenyl-2-picryl-hydrazyl (DPPH). The results of TPC and DPPH obtained in this study are presented in Table 15.6. In Table 15.7 are the results of the statistical analysis. Comparing the same fraction between the two GP varieties, the TPC had very significant differences ($p < 0.01$) with higher content in the stalk and the seed of Loureiro GP than Vinhão (26, 86 and 24, 35.1 mg GAE/g, respectively), while in the skin, the content of TPC was higher in Vinhão than Loureiro (120 and 24 mg GAE/g, respectively) with very significant differences ($p < 0.01$).

Table 15.6. Results of TPC and DPPH in red and white lyophilized GP sample

Type of Sample	Antioxidant activity (mg AAE/g DM)	Total phenolic content (mg GAE/g DM)
BLE	16.1 ± 0.46	26 ± 1.3
BLM	40 ± 1.9	39 ± 1.2
BLP	12.2 ± 0.48	24 ± 1.8
BLG	83 ±17	86 ± 9.5
TVE	18.0 ± 0.73	23.9 ± 0.15
TVM	28.6 ± 0.84	57 ± 1.1
TVP	76 ± 7.2	120 ± 1.4
TVG	29 ± 1.4	35.1 ± 0.97

Table 15.7. Statistical analysis for the TPC and DPPH results (*p* value)

Fractions	Antioxidant activity (mg AAE/g DM)	Total phenolic content (mg GAE/g DM)
BLE vs TVE	0.076	0.008
BLP vs TVP	0.051	0.0003
BLG vs TVG	0.001	0.0004

Figure 15.5 and Figure 15.6 show the correlation between the results of TPC and DPPH with an r = 0.988 for red Vinhão and an r = 0.975 for the white Loureiro.

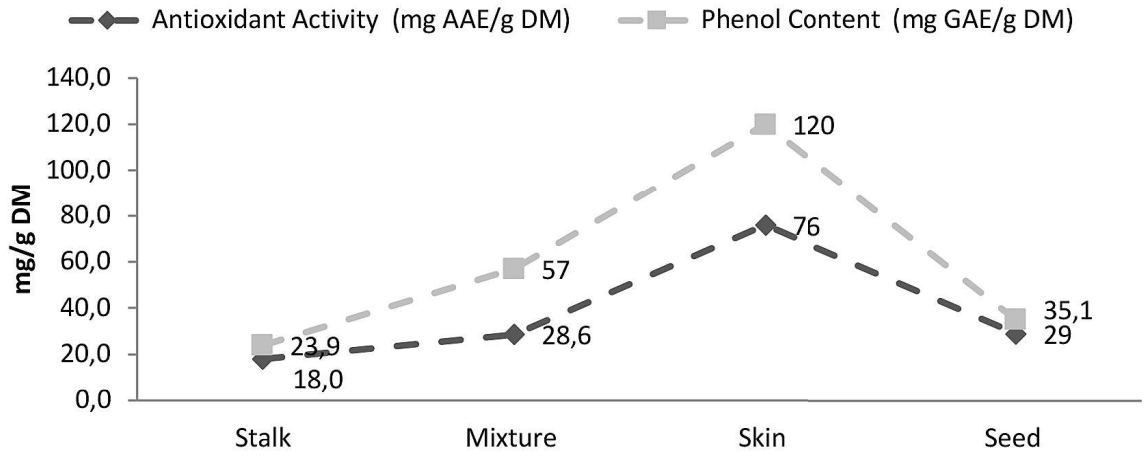


Figure 15.5. Correlation between the TPC and the antioxidant activity in red grape pomace

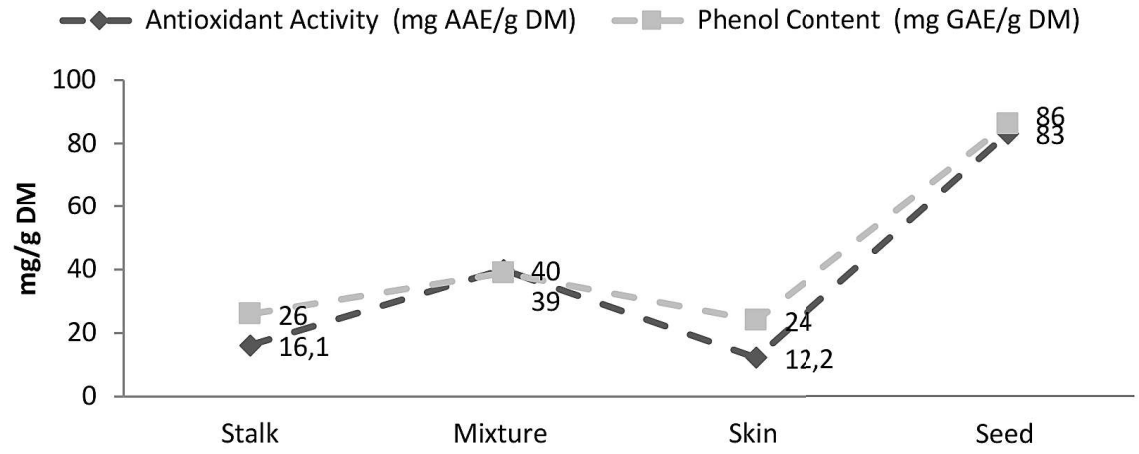


Figure 15.6. Correlation between the TPC and the antioxidant activity in white grape pomace

Comparing these results with other authors (Table 15.8), the values for the seed from Loureiro are similar to Friuli Venezia Giulia but the results of the skin from

Vinhão are higher than all the other varieties. The TPC of the mixture of these two varieties has a range of 39-57 mg GAE/g DM which is between the range of 26.3–131.7 mg GAE/g DM from the bibliography.

Table 15.8. Results for total phenolic content (TPC) in white and red GP varieties from other studies

TPC mg/g DM					Reference
Red varieties	Stalk	Mixture	skin	seed	
Pinot noir	–	67.74	–	–	(Tseng Angela, 2013)
Manto negro	116	26.3	–	–	(Llobera A., 2007)
Friuli Venezia Giulia	–	–	–	51	(Spanghero M., 2009)
California	–	–	–	64	(Spanghero M., 2009)
Pinot Noir	–	56	–	–	(Winkler Anne, 2015)
Touriga Nacional	–	131.7	–	–	(Tournour Hernán H., 2015)
Touriga Franca	–	100.1	–	–	(Tournour Hernán H., 2015)
Tinta Roriz	–	69.3	–	–	(Tournour Hernán H., 2015)
Cabernet S.	–	–	26.7	–	(Deng Qian, 2011)
Merlot	–	–	25	–	(Deng Qian, 2011)
Pinot Noir	–	–	21.4	–	(Deng Qian, 2011)
Agiorgitiko	–	54.02	–	–	(Canellas, 2008)
Kadarka	16–19.4	–	3.2–3.8	35–43	(Llobera A., 2007)
Negro Amaro	–	41.9	33.3	85.8	(Llobera A., 2007)
Dornfelder	–	57	–	–	(Winkler Anne, 2015)
Portugais bleu	–	65	–	–	(Winkler Anne, 2015)
White varieties	Stalk	Mixture	Skin	seed	
Friuli Venezia Giulia	–	–	–	90	(Spanghero M., 2009)
Riesling	–	44	–	–	(Winkler Anne, 2015)
Pinot blac	–	54	–	–	(Winkler Anne, 2015)
Muller Thurgau	–	–	15.8	–	(Deng Qian, 2011)
Morio Muscat	–	–	11.6	–	(Deng Qian, 2011)
Chardonnay	–	38.91	–	–	(González-Centeno M.R, 2013)
Macabeu	–	30.93	–	–	(González-Centeno M.R, 2013)
Parellada	–	46.54	–	–	(González-Centeno M.R, 2013)
Premsal Blanc	–	36.39	–	–	(González-Centeno M.R, 2013)
Premsal Blanc	87.3	34.9	–	–	(Canellas, 2008)
Roditis	57.98	48.26	–	–	(Canellas, 2008)

3.1.3. Results of high heating value (HHV) with a constant volume bomb calorimeter

In order to investigate the possibility of energy recovery for grape pomace, it was determined the heating value of stalks and from the mixture GP from the Loureiro and Vinhão varieties, in the original GP samples. This property was also studied in the extracted samples, to compare the results before and after the extraction of the bioactive compounds. The results are presented in Table 15.9.

Table 15.9. High heating values for studied GP varieties in DM and other biomass values

Sample	HHV cal/g	HHV MJ/kg	Fuel*	HHV MJ/kg
BLE	4,289 ± 26	18.0 ± 0.11	Corn stalks/stover	17.6–18.5
EBLE	4,103 ± 34	17.2 ± 0.14	Sugarcane bagasse	17.3–19.4
BLM	5,043 ± 32	21.1 ± 0.13	Fruit pits	15.8–20.5
EBLM	4,321 ± 49	18.1 ± 0.20	Miscanthus	18.1–19.6
BLP	–	–	Eucalyptus	19.0–19.6
BLG	–	–	Hardwood wood	18.6–20.7
TVE	4,204 ± 20	17.6 ± 0.08	Softwood wood	18.6–21.1
ETVE	4,040 ± 86	16.9 ± 0.36	Coal (wet basis)	23.97
TVM	4,957 ± 26	20.8 ± 0.11	Petroleum coke	31.31
ETVM	2,432 ± 40	17.7 ± 0.17	Crude oil	45.54
TVP	–	–	Natural gas	52.22
TVG	–	–	Hydrogen	142.18

* From website (Boundy B., 2011).

Table 15.10. Statistical analyses for HHV before and after extraction of polyphenols (*p* value)

Fractions	HHV
t-test	
BLE vs EBLE	< 0.0001
BLM vs EBLM	< 0.0001
TVE vs ETVE	0.006
TVM vs ETVM	< 0.0001

In Table 15.9 are presented the results for high heating values from Loureiro and Vinhão GP and some other biomass samples. The results showed that there

were very significant differences between the two varieties, for all the fractions ($p < 0.01$) (Table 15.10). Comparing high heating value for stalks and mixture the later has the highest values (21.1%) and very similar to the study of Toscano that found a heating value for grape mixture of 20.1 MJ/kg and for the stalks of 16.8 MJ/kg (Toscano G., 2013). Comparing the results for the heating value of the wood pellet from Table 15.9 it can be observed that the GP samples even after the fat and the polyphenol extraction have heating values near to the wood pellets, which indicates that this biomass has high potential for energy recovery. Both mixture and stalks from the two varieties have similar values after the extractions (Figure 15.7).

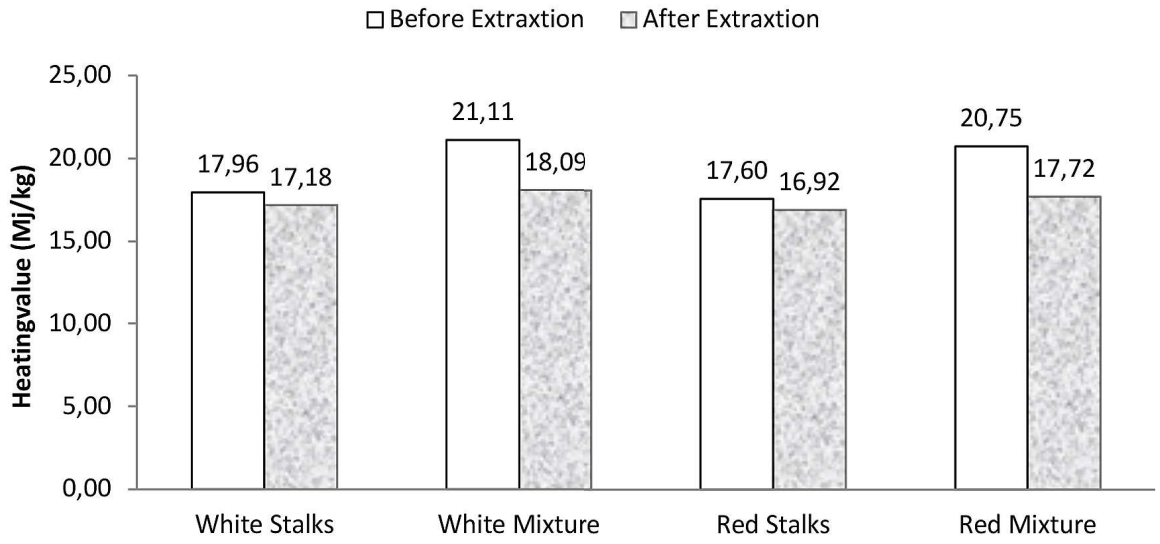


Figure 15.7. Correlation between the heating value (MJ/kg) of the original and extracted sample

4. Brief considerations for the grape pomace applications and conclusion

In conclusion, it can be said that grape pomace cannot be considered anymore as a winery waste but as a by-product, which could extend the grape's commercial life cycle. The separation of GP into different components can be useful to give a distinct value to each part according to its typical characteristics.

The results indicate a high content of oil, 16.4% and 15.7% w/w DM in Vinhão and Loureiro GP, respectively. The high content of total phenolic compounds (TPC) makes them excellent candidates as food additives, which provide food commodities with high antioxidant activity, until 83 mg AAE/g DM in Vinhão

seeds and 76 mg AAE/g DM in Loureiro variety skin, which makes both components of GP great candidates for use as food additives due to their high content.

When comparing the high heating values for stalks and the GP combination, the latter has the greatest values (21.1 MJ/k), which are quite similar to the results of other studies here mentioned and the heating value of the wood pellet. It can be shown that the GP samples still exhibit heating values close to the unextracted GP of 18.1 MJ/k, indicating that this biomass has a strong potential for energy recovery, even after the fat and polyphenol extraction. After the extractions, the mixes and stalks from the two grape varieties have comparable heating values.

Finally, the obtained results on the energy value and chemical composition of GP provided guidance for developing innovative industrial applications. Considering the amounts of GP that are produced annually in Portugal and the chemical properties of GP, it is evident that this sub-product from the winery industry provides new perspectives on potential profitability with alternative industrial applications. Thus, recycling winery by-products constitutes an opportunity for providing valuable materials, contributing to reducing costs and the environmental impact linked to the disposal of these by-products in the production areas.

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