

**MESTRADO**

CONTROLO DA QUALIDADE

**Grape (*Vitis vinifera* L.) by-products: analysis of winemaking industry wastes and possible valorisation**

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analysis of winemaking industry  
wastes and possible valorisation**

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Grau de Mestre em Controlo da Qualidade

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Engenheiro Luís Simões

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É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTA DISSERTAÇÃO APENAS  
PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO  
INTERESSADO, QUE A TAL SE COMPROMETE.

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## RESUMO

Os resíduos provenientes da agricultura e da indústria alimentar constituem um problema crescente com impacto negativo na economia, no ambiente e na saúde humana. A indústria vitivinícola produz uma grande quantidade de resíduos. A gestão destes resíduos poderá constituir um problema ambiental devido ao facto de serem produzidos sazonalmente, ou seja, durante a época da vindima, e possuir características que contribuem para poluição ambiental. O bagaço, o engaço e as borras são os principais subprodutos e resíduos gerados.

Tendo em conta os conceitos de economia circular, os resíduos produzidos no setor vinícola deverão ser reaproveitados, isto é, os compostos bioativos presentes nestes subprodutos depois de extraídos poderão ser utilizados na indústria alimentar, cosmética e farmacêutica. Deste modo, esta estratégia proporciona benefícios tanto aos produtores, com a redução dos custos do tratamento dos resíduos e a possibilidade de um rendimento extra, como também aos consumidores e outras indústrias, permitindo o aproveitamento de compostos bioativos.

O objetivo deste trabalho consistiu na caracterização nutricional, determinação dos compostos fenólicos totais e dos flavonoides e determinação da atividade antioxidante dos dois subprodutos, o bagaço e o engaço. Adicionalmente, este estudo compara os valores obtidos entre as castas em estudo (Touriga Nacional, Alicante Bouschet, Aragonez, Syrah, etc.) e entre as regiões do Douro e do Alentejo.

A composição nutricional de bagaço, um dos resíduos maioritários produzidos durante a vindima, consiste nomeadamente em carboidratos, cerca de 74 g/100 g de peso seco, seguido de proteína (11,46 g/100 g), gordura (8,47 g/100 g) e cinzas (6,02 g/100g). Relativamente ao engaço, que constitui 2 - 12% dos resíduos formados, as proteínas, a gordura e as cinzas encontram-se em quantidades inferiores, aproximadamente 4,06, 7,19 e 0,86 g/100 g em peso seco, respetivamente. Sendo que, a composição nutricional do engaço constitui sobretudo carboidratos (88 g/100 g em peso seco).

Os compostos fenólicos totais e a atividade antioxidante (TPC, TFC, FRAP, DPPH•) foram determinados por métodos espectrofotométricos. Tanto o engaço como o bagaço possuem grandes quantidades de compostos fenólicos totais, com valores de 49,02 – 70,16 mg GAE/g para o engaço e 21,96 – 48,68 mg GAE/g para o bagaço. O conteúdo total de flavonoides do engaço e bagaço foi de 27,76 – 46,93 mg CE/g e de 14,85 – 34,92 mg CE/g, respetivamente. E, por sua vez, a atividade antioxidante era superior no

engação quando comparado com o bagaço em ambos os métodos FRAP e DPPH•. Segundo o método FRAP, os valores das amostras de engação foram de 646,45 – 1094,75  $\mu\text{mol FSE/g}$ , enquanto para o bagaço 324,96 – 750,58  $\mu\text{mol FSE/g}$ . Através do método DPPH•, os valores obtidos foram de 90,25 – 134.03 mg TE/g para o engação e 37,52 – 88,35 mg TE/g para o bagaço.

Em conclusão, o principal objetivo desta dissertação consistiu na partilha do conhecimento e na sensibilização dos produtores, consumidores e a população no geral para o problema da sustentabilidade da indústria vitivinícola e o enorme potencial dos seus resíduos. Este trabalho demonstrou que estes resíduos possuem vários compostos que poderão ser reutilizados noutras indústrias e por isso, contribuir para a redução dos resíduos, diminuindo o impacto ambiental, reduzindo os custos de produção e oferecendo novas vias para a diversidade de produtos.

### **Palavras-chave**

Subprodutos do vinho, engação, bagaço, compostos fenólicos, atividade antioxidante, composição nutricional

## ABSTRACT

Food and agricultural wastes represent a growing concern with negative effects on the economy, environment, and human health. The wine industry produces a significant quantity of residues, whose management and disposal are an environmental burden due to their seasonal character and polluting characteristics. The main solid by-products and residues generated are grape stalk, grape pomace, and wine lees.

From a circular economy perspective, these winemaking wastes could be exploited by extracting bioactive compounds with high added value to be used in the food, cosmetics, and pharmaceutical sectors. As a result, the application of a recovery strategy presents various benefits; producers get advantages from decreasing waste disposal costs and the opportunity for extra incomes, and consumers and industries profit from applying some bioactive compounds.

The main goal of this dissertation was the nutritional and antioxidant characterization of the major winemaking industry wastes, such as grape stalks and pomace. Moreover, this work compares the values obtained regarding different grape varieties (Touriga Nacional, Alicante Bouschet, Aragonez, Syrah, etc.) and regions of Alentejo and Douro.

Grape pomace is one of the major solid by-products generated and consists of fruit skins, seeds, and in certain cases, some stems left over after the crushing and pressing stages of wine production. It contains around 11.46 g/100g dw of protein, 8.47 g/ 100g dw of fat, and 6.02 g/ 100 g dw of ash. Nevertheless, carbohydrates are the main compounds in grape pomace (on average 74 g/100 g dw). On the other hand, grape stalks come from the destemming phase, constituting 2 to 12% of the waste obtained in a winery. They are composed mainly of carbohydrates (roughly 88 g/100 g dw). Protein, fat, and ash are found in minor quantities, around 4.06, 7.19, and 0.86 g/100 g dw, respectively.

Grape pomace and, to a lesser extent, grape stem are well known for their high phenolic content and antioxidant activity. Phenolic compounds and antioxidant activity (TPC, TFC, FRAP, DPPH•) were determined using spectrophotometric methods. Both by-products showed high values of total phenolic compounds, around 49.02 – 70.16 mg GAE/g dw for grape stalk and 21.96 – 48.68 mg GAE/g dw for grape pomace. The total flavonoid content for grape stalk and pomace was about 27.76 – 46.93 mg CE/g dw and 14.85 – 34.92 mg CE/g dw, respectively. Moreover, both by-products showed antioxidant activity, but grape stalk showed higher results than the grape

pomace samples for all antioxidant parameters (FRAP and DPPH• methods). For instance, according to the FRAP method, the values obtained for grape stalk samples were 646.45 – 1094.75  $\mu\text{mol FSE/g dw}$ , while for grape pomace were 324.96 – 750.58  $\mu\text{mol FSE/g dw}$ . According to the DPPH• method, the values of 90.25 – 134.03  $\text{mg TE/g dw}$  were obtained for grape stalk, and 37.52 – 88.35  $\text{mg TE/g dw}$  for grape pomace.

To conclude, the main goal of this dissertation was to share knowledge and sensitize winemakers, consumers, and the population in general to the sustainability problem of this industry and the huge potential of winemaking residues. It shows that the wine industry is provided with natural compounds that may be used in other industries and therefore contribute to reducing winery residues, improving environmental aspects, reducing production costs, and offering new ways to diversify production.

### **Keywords**

Winemaking by-products, grape stalks, grape pomace, phenolic compounds, antioxidant activity, nutritional composition

## **PUBLICATIONS AND COMMUNICATIONS**

Parts of this dissertation were presented in national scientific congresses and meetings:

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## ABBREVIATIONS

AB	Alicante Bouschet
AOAC	Association of Official Agricultural Chemists
CE	Circular economy
DF	Dietary fiber
DPPH•	2,2-diphenyl-1-picrylhydrazyl radical
dw	Dry weight
EMDE	Emerging market and developing economies
EU	European Union
FAO	Food and Agriculture Organization
FIVS	International Federation of Wine and Spirits
FRAP	Ferric reducing antioxidant power
FSE	Ferrous sulphate equivalents
GAE	Gallic acid equivalents
IDF	Insoluble dietary fiber
IPCC	Intergovernmental Panel on Climate Change
ISO	International Organization for Standardization
LAC	Latin America and the Caribbean
MNA	Middle East and North Africa
OIV	International Organization of Vine and Wine
SAR	South Asia
sd	Standard deviation
SDF	Soluble dietary fiber
SDG	Sustainable Development Goals
SSA	Sub-Saharan Africa
TDF	Total dietary fiber
TE	Trolox equivalents
TF	Touriga Franca
TFC	Total flavonoid content
TN	Touriga Nacional
TPC	Total phenolic content

## **Chapter I**

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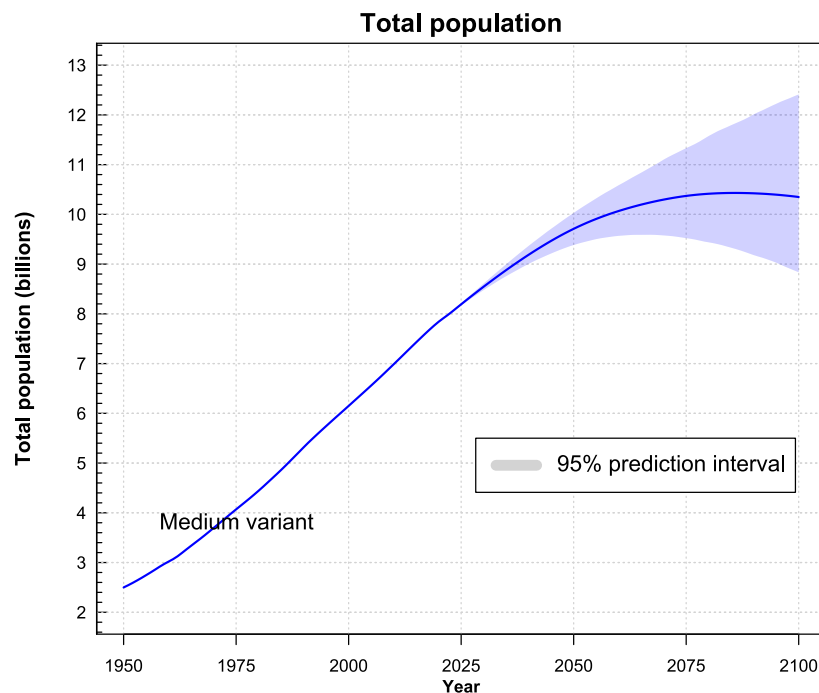
### **Introduction**

## 1. Food supply chain current challenges

Currently, the food supply chain faces several challenges: population growth, climate change, ongoing war, and even pandemics. Therefore, sustainable measures should be adopted to overcome these challenges.

### 1.1 Population growth and climate change

The past century has been marked by increased food production in response to the continuing population and consumption growth. Modern agriculture now feeds 7.8 billion people [3]. For instance, global cereal production has tripled in the past 60 years, allowing a decrease in the proportion of the world's people that are hungry and improving nutrition, despite the total population growth [4]. Unfortunately, the conjecture that hunger would further decline did not come to pass as the impact of the COVID-19 pandemic and the war in Ukraine increased the inequalities [5], as discussed in the next section.



**Figure 1:** Total population growth and prediction to 2022 – 2100 [6].

According to the United Nations, the global population is about to reach 9.7 billion in 2050 (Figure 1), which means that the global food demand will increase further [3, 7]. This will raise several challenges as there is a necessity to increase and sustain food production to match the rapidly changing demand for food and, at the same time, do so in ways that do not compromise environmental integrity and public health [8, 9]. Indeed, feeding nutritiously an increasing population using essentially the same amount of agricultural land

while maintaining soil health and reducing fresh water represent an enormous challenge. This challenge gets trickier when considering that climate change affects food production.

Climate change refers to a change in the state of the climate that can be identified by using statistical tests and identifying the alterations that persist for an extended period of time, typically decades or longer [10]. Climate change implies not only increased average global temperature, but also include more frequent natural disasters such as hurricanes, floods, tornados, etc., and extreme weather events, increased frequency of heavy precipitation, acidification of water, extended dry periods, and potential sea-rise levels [11]. Indeed, according to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), a 1.7 to 4.8 °C increase in the global surface temperature is expected by the end of the 21<sup>st</sup> century [12], and carbon dioxide (CO<sub>2</sub>) levels in the atmosphere have increased from about 284 ppm in 1832 to 415 ppm in 2021 [13]. In particular, the Mediterranean area is projected to suffer higher temperatures, lower precipitation, and increased drought frequency in the future [12, 14, 15]. Portugal, located in Southwestern Europe, will significantly suffer from climate change, bringing new challenges to the food supply chain [15, 16].

The implications mentioned above of climate change certainly will affect agricultural, fishery, and livestock production [11]. The agriculture sector retains almost 40% of the terrestrial surface, so it will probably be the most vulnerable to climate change. It is already reported that a decrease in the world mean yields of maize (4.1%), wheat (1.8%), and soybeans (4.5%) due to climate change [17]. Therefore, producing more food from the same land area while reducing the environmental impacts is a greater challenge. Overcoming this challenge requires not only changes in the way food is produced but also in the way food is processed, distributed, stored, and accessed. Moreover, the most recent coronavirus outbreak and war in Ukraine have exposed the food sector's weaknesses; namely, it has generated a significant food crisis and insecurity for a growing number of people unable to afford their basic daily food [18, 19].

According to the Food and Agriculture Organization of the United Nations (FAO):

*"Food security exists when all people, at all times, have physical and economic access to sufficient, safe, and nutritious food that meets their dietary needs and food preferences for an active and healthy life."*

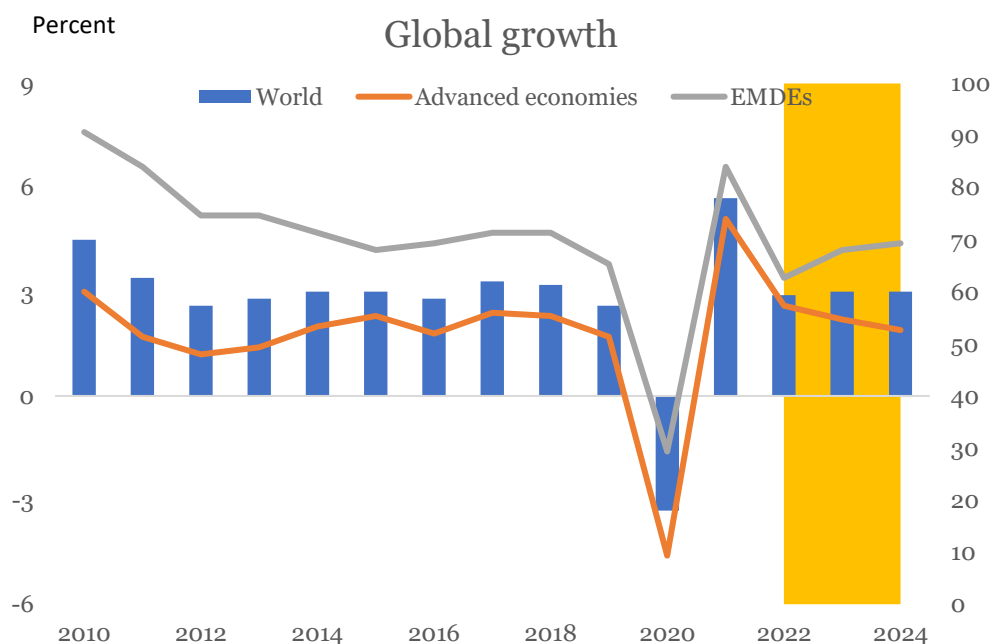
To be food secure, a population, family, or individual must always have access to sufficient food. This access can be compromised because of cyclical events (e.g., seasonal food insecurity) or as a consequence of sudden shocks such as economic, climate crises, pandemics, or war crises currently experienced [4]. Therefore, to ensure food security, a

multifaceted and linked global strategy is required. There are a broad range of options, scientific and technological innovations in the food system that need to be pursued simultaneously [7, 8]. For instance, reducing waste, changing diets, increasing production limits, and closing the yield gap are some of the topics that have been addressed by the scientific communities to not simply to maximize productivity but move forward towards sustainability and food security.

## 1.2 COVID-19 pandemic

Corona Virus Disease 2019 (COVID-19) has brought a global tragedy not only for human lives but also for economic activities like logistics, supply chain, manufacturing operations, and several other areas [20]. The COVID-19 pandemic has severely impacted all the industrial sectors worldwide, including the food industry. Figure 2 demonstrates the largest economic shock that the world economy has witnessed during the pandemic, causing a collapse in global activity [2]. Moreover, the global economy is experiencing another major negative shock, the Russia’s invasion in Ukraine, that magnify pre-existing strains from the pandemic, as will be further discussed in the next section.

In the early stages of the COVID-19 pandemic, general panic resulted in empty supermarket shelves, and lack of non-food items and key food. Rice, flour, pasta, frozen foods, canned goods, bottled water, toilet paper, hand soap, and hand sanitizers were some of the goods that was hard to find [21].



**Figure 2:** Global growth, advanced economies growth, and emerging markets and in developing economies (EMDEs) growth [2].

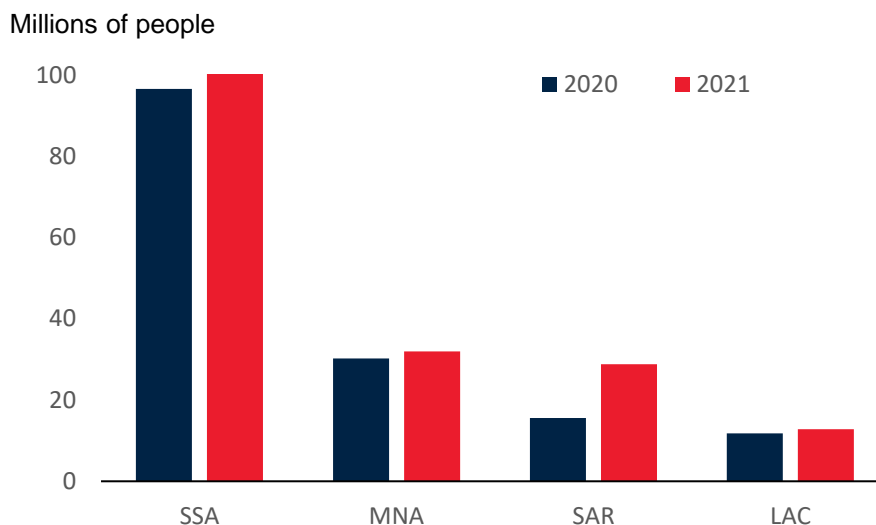
Note: Shaded area indicates forecast.

The food system includes a complex of closely correlated stages of food production and safety of the final products, such as processing, distribution, consumption, and waste management activities, all severely affected during the pandemic [19]. For instance, not only the panic buying but also the labor shortages due to worker self-isolation, illness, or movement restrictions, had a significant impact on the disruption of the food supply chain. On the other hand, during the pandemic, millions of people have found themselves unemployed, and according to the estimations, approximately 49 million people were predicted to fall into poverty in 2020 [2, 22]. The loss of income, food shortages and the boost of food prices exacerbated the inequalities across and within the countries and have led millions of people to food insecurity [2, 5]. Indeed, it is estimated that between 702 and 828 million people were affected by hunger in 2021, the number has grown 150 million since the outbreak of the COVID-19 pandemic [5].

To conclude, the COVID-19 pandemic has highlighted the necessity to build a robust food security system and adopt principles of sustainability to be able to respond not only to the consumers' needs but also to the requirements of the producers, the supply chain, waste management, and the economic environment [18, 19]. Therefore, it is crucial that academic researchers and food sector experts start to think about how to ensure food security, reduce waste of food and the costs of food waste recovery, their possible reutilization in the food chain, as well as find alternatives that promote health and support the population immune system, developing functional foods fortified with antioxidants and bioactive compounds and identifying alternative and safe protein sources [8, 18].

### **1.3 War in Ukraine**

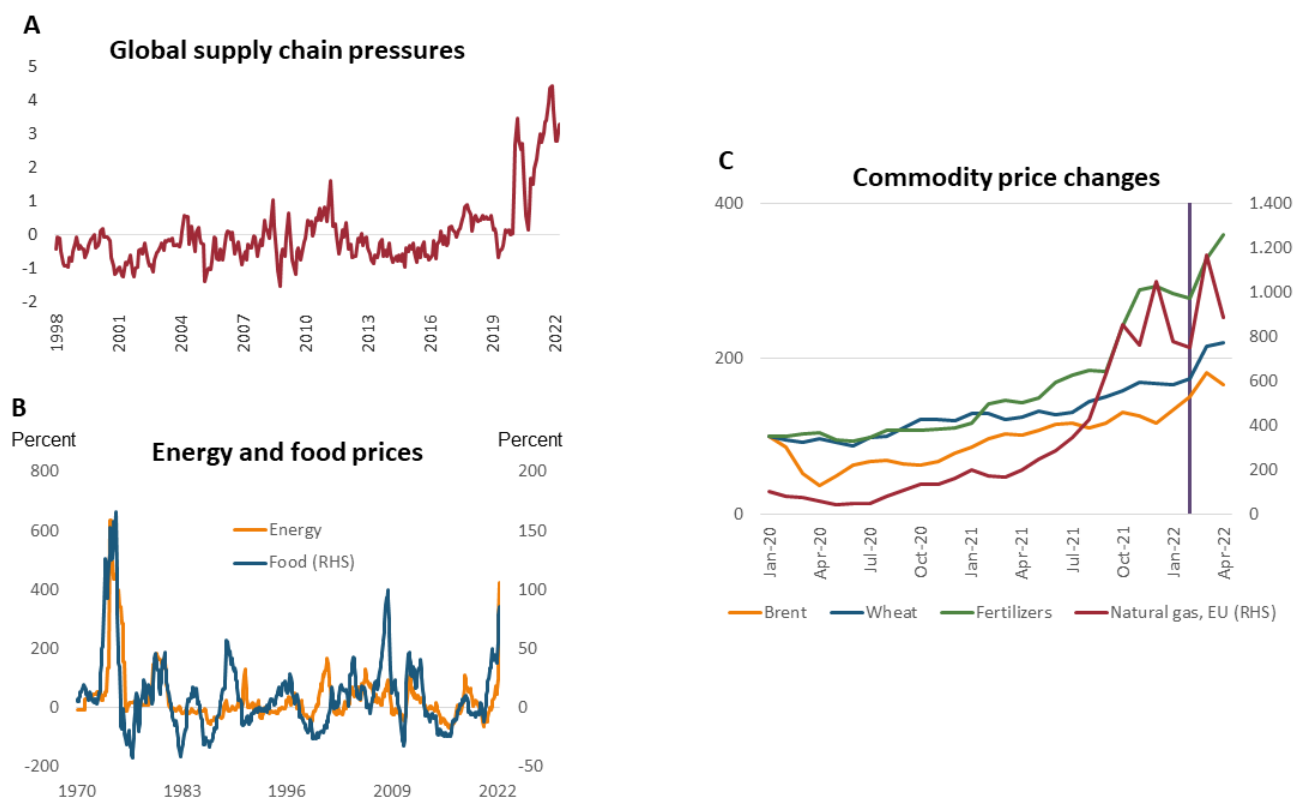
After more than two years of the pandemic, the Russian Federation's invasion of Ukraine had a major global effect on supply chains, commodity markets, financial conditions, and inflation, contributing to the slowdown in global growth [2, 23]. Moreover, the war in Ukraine is leading to a significant increase in agricultural commodity prices in addition to supply disruptions, thus intensifying food insecurity and poverty [2, 24]. According to the World Bank Flagship report, the lasting effects of the pandemic combined with the war and the rise in food prices will lead to a net increase of 75 million people in extreme poverty by the end of this year compared with the pre-pandemic projection [2].



**Figure 3:** Number of people in acute food insecurity (adapted from [2]). Note: LAC = Latin America and the Caribbean, MNA = Middle East and North Africa, SAR = South Asia, SSA = Sub-Saharan Africa.

The Russian Federation and Ukraine are among the most important producers of agricultural commodities in the world, particularly wheat, sunflower, sunflower oils, maize, and fertilizers. Before the crisis, the two countries together produced nearly 30% and 20% of the world’s traded wheat and maize, respectively [25]. Moreover, they accounted for almost 80% of global exports of sunflower seeds [23], and the Russia Federation is the main exporter of phosphorous fertilizers, potassium, and nitrogen [2]. As a result, the COVID-19 pandemic and now the war and sanctions, the prices of major food commodities and fertilizers have skyrocketed (Figure 4C), affecting all countries, and especially the ability to meet the needs of consumers of vulnerable food-importing countries [24-26]. Global trade slowed in 2022 as the result of the increasing pressure in supply chain, affected by persistent effects of the pandemics as many key cities of China are still in lockdown, and physical and logistical dislocation induced by Ukraine invasion by Russia (Figure 4A). The resulting increase of the food and energy prices (Figure 4B) are likely to lower real per capita incomes in many countries and substantially worsen global food insecurity and poverty [23].

It is urgent to gather all the novel ideas for ensuring food security, promote the efficient implementation of these ideas and emphasize the importance of having a resilient and robust food system.



**Figure 4:** Global supply chain pressure index (A). Energy and food prices (B). Commodity price changes, monthly data, last observation is April 2022. Purple line denotes the onset of the Russian Federation’s invasion of Ukraine (C). [2].

## 2. Agro-industrial by-products within the food supply chain

In 2015, the members of the United Nations adopted an agenda: “The 2030 Agenda for Sustainable Development”, which has a total of 17 Sustainable Development Goals (SDGs), being some of them “no poverty (SDG1)”, “zero hunger (SDG2)”, and “climate action (SDG13)” [27]. The FAO report dispels any lingering doubts that the world is moving backward in its efforts to end food insecurity, hunger, and malnutrition, with only eight years away from 2030, the SDG target year [5]. This means that the food supply chain should adopt more sustainable measures to preserve natural resources and guarantee food for everyone. Therefore, food waste management could be an important tool to combat hunger, maximize the benefits to the environment, society, and economy, and improve food security in the world’s poorest countries.

Agriculture and food production produces large amounts of by-products and wastes (500 million tons), followed by storage and managing (350 million tons) and consumption (340 million tons) [28]. There is increasing interest in the valorisation of by-products produced at different points and stages of the production chain in the food industry [29]. At the state

and local government levels, discussions have taken place on several occasions about the accumulation of by-products and their possible negative environmental impacts, as most of them are discarded in landfills or incinerated [29, 30]. The sustainability and environmental safety of the current strategies for food waste management is questionable and constitute a severe problem from an environmental and economic point of view [31].

One of the sustainable strategies for industrial waste management consists in reuse of these residues. Indeed, the circular economy concept from the economic point of view, has been advocating suitable strategies to valorise and manage these secondary products, promoting environmentally friendly solutions and bioeconomy growth in different industries [32]. Thus, an integral valorisation of food production secondary products could lead to significant improvements, as these are often characterized by a high content of value-added bioactive compounds, which could be extracted and used as novel industrial materials [33, 34]. Novel food ingredients, products, cosmetic or pharmaceutical formulation may emerge and thus support the valorisation of by-products in the food chain, generating income streams, economic prospects, and employment opportunities [35]. Indeed, by-products can be used in different processes of the same industry or sold to other industries. There is an increased interest in high-value compounds, such as dietary fiber, phenolic compounds, proteins, etc., that can be extracted and reused as novel ingredients [33, 36, 37].

### **3. Wine industry**

Grapes are one of the largest fruit crops in the world, with 77 million tonnes produced in 2019 [38], the vast majority of which was used for the production of 260 mhl of wine [1]. Several European countries, such as - Italy, France and Spain, are the major producers of wine, with an annual contribution of around 59% of global wine production [39]. Portugal is the 11<sup>th</sup> largest producer of wine and the 9<sup>th</sup> exporter worldwide [1, 40]. Because of the size of the wine agro-industrial sector and the amount of land devoted to the production of wine, one of the main economic and environmental problems is associated with high amounts of wine by-products and wastes produced [40-42]. The potential value of these by-products is frequently lost as they are often underexploited. However, they have very interesting bioactive compounds and can be transformed into value-added products, applying the circular economy principles, thus avoiding sending them to disposal or their incineration [39, 40, 43]. This replaces the end-of-life idea of a by-product adding value to it, involving a better environment and economic balance, and may even result in a new ingredient or food.

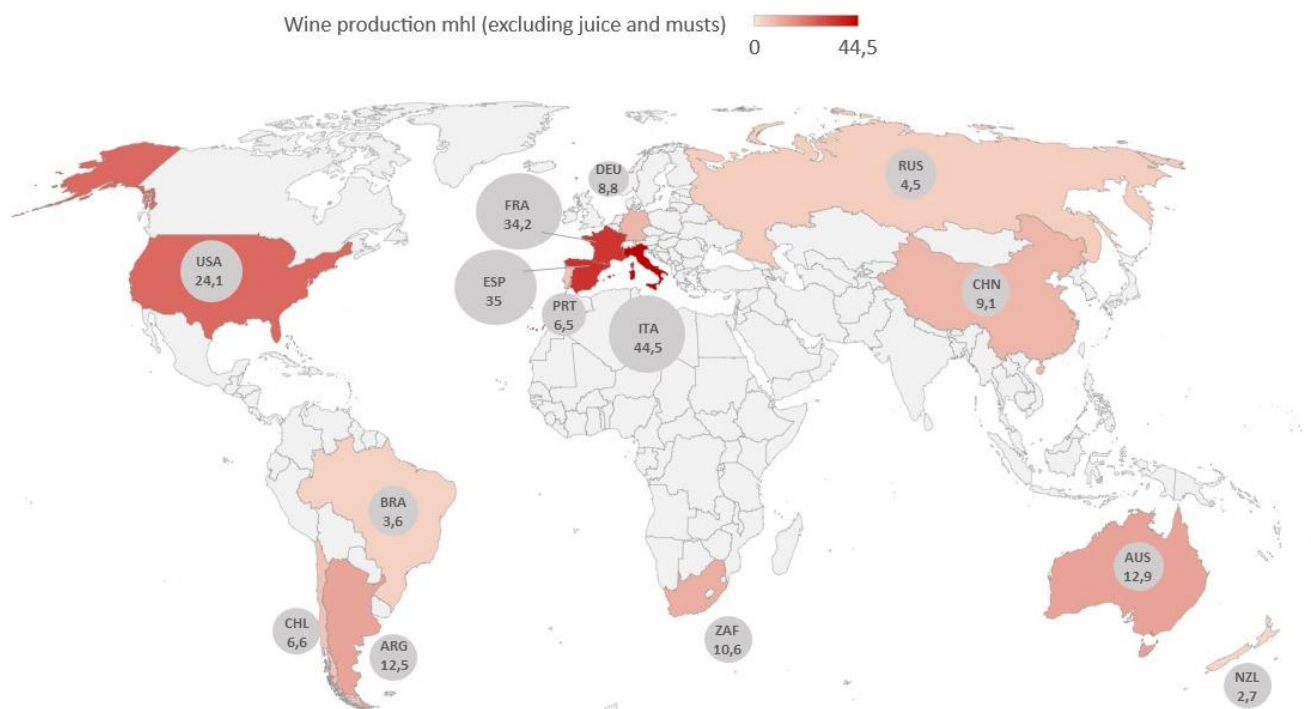
This work describes the different by-products generated in the winery, and their chemical composition and reviews current studies on their valorisation from an economic point of view and scientific, environmental, and social to make this lucrative and part of the overall business of wine production.

### **3.1 Wine consumption**

The culture of wine can be traced as far as written records exist, pointing at Egypt, Greece, and Rome as the first to have a culture of wine, although archaeological evidence indicates that wine can be traced back as far as early Neolithic times [44, 45]. Wine affected living in many ways, initially was used locally not only as a beverage but also for religious and medical purposes, and soon enough traded through the Western World [45, 46]. In Europe, particularly in France and Italy, the higher quality wines based on intrinsic advantages such as climate, soil, and winemaking practices, have establish themselves since the Middle Ages and particularly during the 19<sup>th</sup> century, contributing to creation of the *terroir*<sup>1</sup> concept [44, 45]. Nowadays, the rising of new competitors out of the old continent and the continuous change in consumers' behaviours force the wine industry to undergo intense evolution [47]. "Old World" countries such as Italy, France, Spain, Germany, and Portugal are responsible for the majority of grapes and wines produced [48]. These markets also concentrated most of the consumption, as in these countries, wine was widely considered part of the traditional way of life. However, over the past three decades due to emerging "New World" wine countries, particularly Chile, Argentina, Australia, New Zealand, the USA, and South Africa, the traditional wine consumption pattern has been challenged (Figure 5) [1, 44, 48].

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<sup>1</sup> Terroir comes from the French *terre*, the earth or dirt in which the vine grows, and the notion of *terroir* refers to the soil, exposure, slope, and other physical characteristic of a vineyard.



**Figure 5:** Wine production (juices and must excluded) in major countries in 2021 (adapted from [1]).

In this context, not only do consumers' taste preferences play a crucial role in the wine industry, but also the change in their behaviour to integrate more sustainable and environmental considerations into their lifestyle choices. Nowadays, more consumers' decisions are based not only on the quality of the product but also on how these products affect their health and environment [47, 49]. Thus, the rise of the "green" consumers is marking a shift in the pattern of consumers purchasing towards increased sustainability.

### 3.2 Wine production

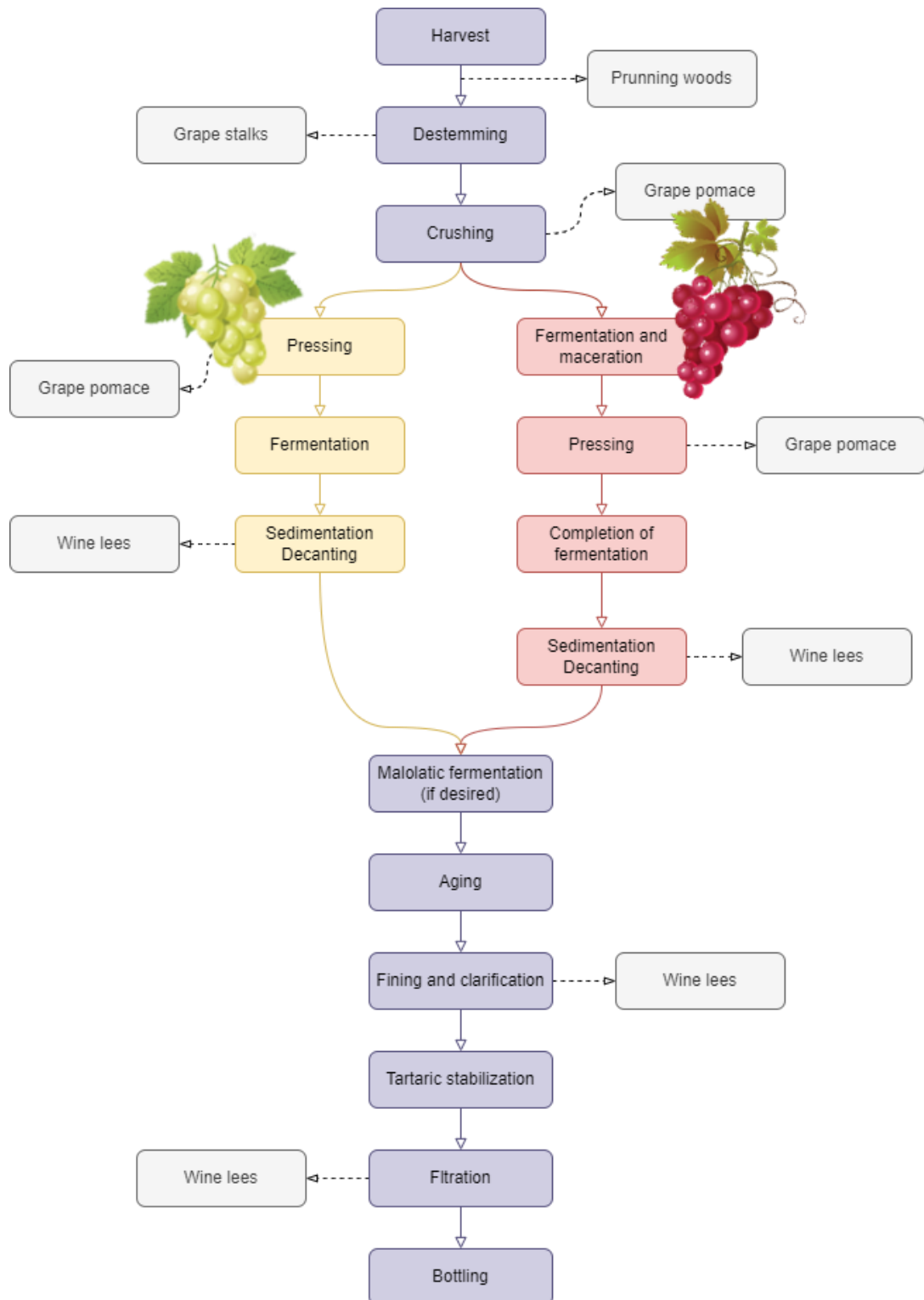
According to the EU legislation (Reg. (EU) 1308/2013), wine is defined as a "*product obtained exclusively from the total or partial alcoholic fermentation of fresh grapes, whether or not crushed, or of grape must*". This winemaking process has been performed several thousand years ago since the age of the first settlements in the Tigris-Euphrates basin [45]. The vine *Vitis vinifera* has been carried from this region to all the Mediterranean countries. Nowadays, different kinds of wine are produced worldwide and establish four main classifications – red, white, rosé and dessert – depending on the vinification process.

Wine harvest occurs from August to October in the northern hemisphere, while in the southern hemisphere, from January to April. Grapes are picked at the right ripening stage for wine production and unloaded in the destemming machine [35]. The grape stem, often referred to as stems, are removed to avoid the formation of an herbaceous taste in the wine

[50]. However, to provide acidity and tannins useful for wine stabilizations parts of the stems may be left in the mass. At this point, the grape juice is extracted through crushing, and the grape pomace residue (frequently referred to as grape marc) is obtained. In red wines and for a few exceptions of white wines, the maceration process occurs, which means that grape pomace is left with the juice (in the winemaking process referred to as must) for a certain period of the fermentation phase in order to enhance the extraction mainly of phenolic compounds and pigments of the grape skins to the must [45, 51]. After the maceration step, must can be removed from the steel container (fermenter), and grape pomace is pressed to obtain additional but lower quality wine and then discarded or sent to the distilleries where it suffers distillation process to produce ethanol. After the completion of fermentation, which usually takes 5 – 21 days, sugars are transformed into alcohol, and the wine lees are generally removed by the decanting process. Depending on the wine type, second fermentation (malolactic) can be performed to convert the malic acid into lactic acid, which reduces the acidity of the wine and gives it a smothering taste [52]. The following steps may vary according to the wine style: aging (wooden barrels, concrete, amphoras, or stainless steel), clarification, filtration, and cold stabilization. Filtration and stabilization steps generate additional residues and wine lees that are discarded.

In the case of white wines (with a few exceptions), grape pomace is discarded immediately after the crushing step. The grape pomace is removed to avoid the transfer of certain compounds to the must, as they are very susceptible to oxidation, negatively affecting color and wine flavor. Another main distinction between the red and white winemaking process is as nearly all red wines go through malolactic fermentation but only a few white wines.

Figure 6 shows a scheme of standard white and red winemaking technology with evidence of the resulting by-products of these processes. Winemaking wastes constitute an environmental problem as they contain a significant amount of organic species with high chemical and biological oxygen demand. In fact, there is growing interest in finding novel management and valorisation strategies for grape by-products that are sustainable and follows the circular economy rhetoric.



**Figure 6:** White and red wine production process and generation of by-products.

### 3.2.1 Viticulture and winery residues

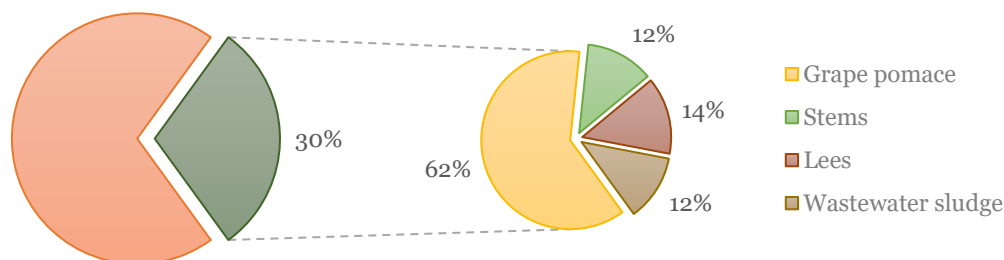
As described previously, wine production generates different residues throughout the production chain characterized by suspended solids and high contents of biodegradable compounds [53, 54].

Table 1 shows the amounts (kg) of each by-product obtained in three consecutive vintages in the Herdade do Peso (one of the wineries that provided the samples for this work) in order to understand which are mostly formed since this will be related to the consequences for the environment. Some differences were observed between the results obtained by different studies, probably due to the test conditions used by different research groups. It is important to mention that the amount of grape stem formed mainly depends on the type of harvest if the grapes are harvested by hand or mechanically. For instance, the harvest in Herdade do Peso is mechanical, so the amount of grape stem residue produced is considerably lower when compared to the manual harvesting.

**Table 1:** Total amount (kg) of each by-product formed in 2019, 2020 and 2021 vintages in Herdade do Peso and the respective percentage.

By-product/ product	2019	2020	2021
Grape	1.290.214 (100%)	1.601.780 (100%)	2.238.180 (100%)
Wine (L)	915.100 (71%)	1.198.780 (75%)	1.648.388 (74%)
Total waste	212.700 (16%)	296.747 (19%)	379.343 (17%)
Grape pomace	186.700 (87%)	212.060 (71%)	307.040 (81%)
Grape stem	6.600 (3%)	6.407 (2%)	13.500 (4%)
Wine lees (L)	20.620 (10%)	78.280 (26%)	59.303 (16%)

In terms of percentage, it is estimated that around 30% (w/w) of the grapes used for winemaking represent solid wastes, of which approximately 62% is grape pomace, 12% stems, 14% lees, and 12% wastewater sludge (Figure 7) [55, 56]. Therefore, the main by-product of the wine industry is the grape marc or pomace, which comprises grape skins, seeds, and in some instances, stems left after the crushing and pressing stages of wine production. Other grape processing by-products include vine shoots (vine pruning), wine lees, filtration residues, and wastewater [53, 57, 58].



**Figure 7:** Approximate distribution of the organic wastes produced in the wine industry.

The generation of different by-products during the red and white wine production process is shown in Figures 6 and 7. For instance, grape pomace is left over after crushing, draining, and pressing and is constituted by peels and seeds. Furthermore, wine lees are a sludge material formed by particles that precipitated at the bottom of wine tanks or barrel or generated in the clarification process of the wine, mainly containing yeast residues and dead yeast. The rest of the wastes are stems, constituted by leaves and branches of the grapevine, and wastewater [52, 55]. The quantity and quality of winemaking waste depend on different production scenarios, such as the type of technology used and the operating practices within a given winery [35]. For example, the investigation by Velázquez-Martí et al. (2011) showed that the quantity of pruning material is strongly influenced by the shape of the structure of the plantation [59].

Various wine companies established different waste management protocols. The current management of these wastes is mainly carried out by external companies, which is an expensive and challenging alternative for the wine industry as transportation is required in a short time, there is low bulk density in the case of the stem, and high disposal costs (incineration) [55]. On the other hand, international legislation is becoming increasingly more exigent in terms of waste disposal and treatment, as the concern about the environment is growing.

The more conventional uses of winemaking residues are summarized in Table 2. For instance, pruning residues are traditionally burned or mulched and left in the field to serve as a source of nutrients and increase the organic matter content since their economic value is minimal. Additionally, these can improve soil structure conditions and texture. However, it is possible that disposal of these pruning residues in the field can negatively impact the soil, as it may occur sorption of residual herbicides, pesticides, and heavy metals. Moreover, incineration of these residues in the field causes significant environmental problems because of the release of toxic compounds like greenhouse gases and polycyclic aromatic

hydrocarbons. In some countries it is prohibited to burn these residues in the field. Incineration of grape stems is also potentially hazardous because of the release of cancerous compounds, so in the alternative, traditionally, they are disposed of by distilleries, landfills, or rural areas [60]. Grape stems can also be used as fertilizer, although they must be conditioned before use due to the presence of polyphenols.

The main fraction of solid wastes, grape pomace, is traditionally distilled to produce alcohol, which is used to make well-appreciated distilled spirits, liqueurs, and liquors, so as to fortified wines [37]. In fact, the European Council Regulation (EC) 1493/1999 on the Common Organization of the Wine Market, reformed in 2008 (EU Reg. No479/2008 and 555/2008) and 2013 (EU Reg. No 1308/2013), established that the Member States may require the delivery to the distillation of a part or of the entirety of the secondary products of the winemaking to be compulsory. This obligation can also be fulfilled by delivering wine to the vinegar industry. The distillation of the by-products is not profitable for the wineries, the earned money is often just enough to pay the transportation costs. Some of the authorized alternatives to the distillation of grape pomace and lees are extraction of tartaric acid, land-spreading (maximum 3 tons/ha), production of fertilizers, and cosmetic and pharmaceutical applications. Grape seed oil production is another common exploitation of the grape pomace, usually carried out on the seeds removed from distilled pomace. One of the grape seed oil production leaders is the Italian Company Tampieri SpA, producing around 250 million liters of oil annually.

**Table 2:** Review of the main conventional uses of winemaking wastes (adapted from [52]).

<b>Residue</b>	<b>Conventional Uses</b>
<b>Vine pruning</b>	Spreading or incinerating in the field
<b>Stems</b>	Landfill animal feed; land-spreading; incineration; compost
<b>Pomace /Skins</b>	Distillation; incineration; colouring agents; land-spreading;
<b>Seeds</b>	Oil recovery; incineration
<b>Lees</b>	Distillation; tartaric acid; colouring agents; nutritional supplements; incinerations; landfill; land-spreading

#### **4. Sustainability and the importance of circular economy in the wine industry**

The wine production chain is inevitably a source of a substantial quantity of waste, which does not have any economic value or any application, and constitute a serious concern when disposed in the environment [61]. Unfortunately, many of these wastes still end up in landfills rather than being treated and used in agriculture or for many other purposes [52, 54].

The ongoing imperative in every industrial sector is currently "to be sustainable," and the wine sector is not an exception to this. Nowadays, the term "sustainability" is commonly used, and a range of definitions are available. In 1987, the United Nations defined:

*"Sustainable development is a development that meets the needs of the present without compromising the ability of future generations to meet their own needs".*

According to the International Organization for Standardization (ISO), the ISO 14000 family provides practical tools for companies and organizations that wish to manage environmental responsibility [62]. The goal of this standard is not only to identify and reduce environmental waste but also elaborate strategies for continual progress in waste reduction. In ISO 14001:2015, "Environmental management systems – Requirements with guidance for use," the definition of sustainability involves not only the environment but also society and the economy in order to accomplish its own needs without compromising future generations [62, 63].

Regarding grape and wine products, the concepts of "sustainability" become less clear. Sustainable viticulture and winemaking lack specific standards and guidelines to support winemakers in the improvement of their sustainability performance [64]. This has encouraged several organizations, mainly the International Organization of Vine and Wine (OIV) and the International Federation of Wine and Spirits (FIVS), to develop sustainability guidelines [65-67].

According to OIV, sustainable vitiviniculture is defined as:

*"Global strategy on the scale of the grape production and processing systems, incorporating at the same time the economic sustainability of structures and territories, producing quality products, considering requirements of precision in sustainable viticulture, risks to the environment, products safety and consumer health and valuing of heritage, historical, cultural, ecological and aesthetic aspects."*

In 2008, OIV approved an implementation guide of principles of sustainable development in the vitiviculture sector, considering equally environmental, economic and social aspects, and FIVS published the Global Wine Sector Sustainability Principles (2016) [65, 66, 68]. Currently, there are many certification standards, regulatory frameworks, and labelling systems regarding various aspects of sustainability. Figure 8 provides an overview of the leading certification in wine.



**Figure 8:** Most commonly used certifications for organic, biodynamic, and sustainable wine worldwide (adapted from [69]).

To address sustainability issues, the concept of the Circular Economy (CE) has been gaining influence. The CE has become a model of production and consumption, which includes sharing, recycling, and reusing existing materials and products, extending their life cycle.

Focusing on the European Parliament's implementation of the concept of CE is described:

*"The circular economy is a model of production and consumption, which involves sharing, leasing, reusing, repairing, refurbishing, and recycling existing materials and products as long as possible. In this way, the life cycle of products is extended."*

In practice, it implies reducing waste to a minimum. Thus, the CE aims to keep a product within the economy even when it reaches the end of its life, designing strategies to give them

a "new life", thereby creating additional value. Thereby, CE model depends on large quantities of easily accessible and cheap materials and energy. To enhance the contributions to sustainable development, in 2018, the ISO/TC 323 Circular Economy was created to allow the development of structures, supporting tools, and guidelines.

Driven by the sustained growth of resource consumption, the CE has emerged as a replacement for the current linear economic development model [70, 71]. Rapid population growth and industrialization, combined with ongoing environmental concerns, energy security, and limited virgin resources, forced the transition to a CE that aims to provide a sustainable alternative to the current "take, make, use, and dispose" paradigm [71, 72]. The CE model simultaneously addresses general concerns around climate change and the sustainable development of private sectors. In fact, regarding the private sector, CE encourages the generation of new business via exploring the additional value and developing secondary products and/or services without additional natural resource consumption. Over the years, the number of scientific research focusing on CE has increased, namely the research concerned with reducing wastes or reusing them to extend their life cycle before disposal. Besides, sustainability studies regarding the evaluation of green technologies for valorisation of industrial by-products are also steadily increasing.

Valorisation of by-products is expected to be at the forefront of a CE due to the abundance of wastes and their negative effects on the environment. Particularly, in the food industry, around 1,3 billion tons per year of food produced worldwide is wasted (the equivalent to 3300 Mtn of CO<sup>2</sup> emissions per year), of which 14% corresponds to the waste from production before reaching the retail level [40, 71, 73]. Wine production, one of the most important agricultural activities throughout the world, is not an exception to this. The wine industry generates different organic and inorganic residues, estimating that the production of 100 Kt of must/wine generates 38.9 Kt of wine grape secondary products [39, 74]. Several published studies have evaluated the impact of winery waste disposal on the environment through carbon footprint assessment. For example, according to Bevilacqua *et al.*, the wine production of 51 mhl results in the emission of 834 000 tons of CO<sup>2</sup> just from pomace [75, 76]. The International Organization of Vine and Wine estimates world wine production at 260 mhl for the 2020/2021 campaign with Europe (Spain, Italy, France, Germany, and Portugal), Australia, America (USA, Chile, and Argentina), and South Africa being the most important wine-producing regions [1, 53]. Consequently, by analogy, the global production of 250 mhl of wine will result in 4 088 235 tons of CO<sup>2</sup> emission just from pomace.

Moreover, a large amount of waste is produced during the short period of harvest, though increasing the concentration per area, which constitutes the main environmental problem

in terms of the management of the winery by-products [33, 77-79]. Discard in land fields or incineration may be detrimental to the environment due to polluting characteristics of these wastes, such as low pH and high content of phenolic compounds, with antibacterial and phytotoxic properties, contributing to slow biodegradation of these wastes [54, 77, 78]. Therefore, these residues may cause environmental problems such as pests, water pollution, bad smells, and oxygen depletion in ground waters and soil by tannins and other compounds, affecting the vegetation and animals around [78]. In this regard, in viticultural regions that generate a large volume of waste and by-products, it is urgent to identify high-added-value ingredients and establish markets to enable their proper distribution.

## **5. Potential of winemaking by-products to be value-added products**

The valorisation process targets the recovery of winemaking by-products for novel product applications and constitute an alternative to reduce environmental impact of wine industry. The valorisation of winemaking by-products could pass through extraction and commercialization of bioactive compounds, such as phenolic compounds, dietary fibers, vitamins, and unsaturated fatty acids, and other compounds with nutritional value as proteins and oil from grape pomace [80]. Thus, the emerging alternative procedures and technological advancement should be applied to recover bioactive compounds from wine industry by-products to promote “greener” and efficient practices [33].

Table 3 summarizes the most researched non-conventional uses of grape by-products. The extraction of bioactive compounds has received much attention, followed by the production of energy, food ingredients, and compost [52]. Food, cosmetics, and pharmaceutical applications of wine industry by-products have been reported in the literature since these ingredients or additives have multifunctional proprieties.

### **5.1 Dietary Supplements**

Winemaking by-products containing biologically active compounds, mainly antioxidants and dietary fiber, have a promising role in the food industry. In the USA market, grape extract, grape seed, and red wine powder are incorporated into several dietary supplements due to their antioxidant, hypoglycaemic, and hypolipemic effects [33]. In Europe, grape seed extracts have also been used mainly for therapeutic purposes, as doctors often prescribe OPCs (Oligomeric Proanthocyanidins) as complementary medicine.

The wine industry by-products contain resveratrol, one of the most known compounds for its biological activity, which is commonly find as ingredient of dietary supplements. For

instance, the food supplement company that produces LifeExtension Resveratrol supplement claims that it "promotes youthful gene expression similar to calorie-restricted diets, encourages healthy insulin sensitivity and mitochondrial function and supports a healthy inflammatory response". In the nutraceutical supplementation, other grape polyphenolics are applied due to their proven antioxidant properties and potential health benefits. For example, in the market, the Lamberts Super Strength Antioxidant Complex provides 10 000 mg oxygen radical absorbance capacity units due to its high polyphenolic content. This supplement is a blend of rosemary, green tea, grape seed, and oregano extracts.

Moreover, antioxidant compounds present in grape seed, such as vitamin E, have crucial role in the protection of lipoproteins and polyunsaturated fatty acids from oxidation by free radicals. It has been shown that this protection relates to the prevention of atherosclerosis, and in animal studies have been demonstrated considerable anti-inflammatory effects when animals diet contained supplementation of grape pomace [36].



**Figure 9:** Examples of dietary supplements containing grape bioactive compounds.

**Table 3:** Applications and potential benefits of winemaking wastes in different industries

By-product	Compound	Possible application	Potential benefits	Reference
<b>Grape pomace</b>	Phenolic extracts	Moisturizing cream	Antioxidant capacity, prevent lipid oxidation and enhance stability (increase shelf-life) Antimicrobial capacity Natural emollients (oils)	[80]
		Yogurt	Antioxidant source	[81]
	Dietary fiber	Cookies/Muffins	An alternative source of DF and phenols	[82, 83]
		Seafood	Increase water retention and oil retention capacity	[82]
		Flour	Fiber supplement	[81, 84]
<b>Grape seed</b>	Protein	Fining agent	Clarification of red wines (alternative plant-based protein)	[85]
	Fatty acids	Encapsulation	Nanoemulsions formed by spontaneous emulsification offer a simple way to encapsulate resveratrol	[86]
<b>Grape stalks</b>	Phenolic acids, flavonoids, and stilbenes	SO <sub>2</sub> substitution	Natural antioxidant and stabilizer	[87, 88]
		Phenolic compounds	Fining agent	Protein precipitation
	Phenolic compounds	Disinfectants	Inhibition of pathogens <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> , <i>Salmonella enterica</i> , and <i>Escherichia coli</i>	[90]
		Antibiotic	Antimicrobial activity Inhibit human clinical pathogens	[91]
	Fiber	Food packaging as foams	Production of bio-degradable packaging Substitute for expanded polystyrene (EPS)	[92]

<b>Wine lees</b>	Proteins	Practical diet for ruminants	Source of protein and energy supply	[93]
		Development of fortified cereal bars	Improving protein content	[94]
	Dietary fiber	Production of high-added value ice cream	Superior structure, high antioxidant effect, oxidation inhibition on human erythrocyte membranes Enhanced physical, chemical, and sensory properties, protection against <i>Lactobacillus acidophilus</i> during storage	[95-97]
	Phenolic compounds	Alternative to synthetic additives	Enhancement of antioxidant and antimicrobial activity in burgers	[98]
		Fortification of dairy products	Protection of probiotics <i>L. acidophilus</i>	[97]

## 5.2 Food Additives and Nutraceuticals

Diverse compounds with different properties constitute the winemaking by-products, thus enabling a wide range of potential functions and technological uses. The main compounds present in these by-products are phenolic compounds with high bioactivity, namely antimicrobial and antioxidant properties, followed by compounds such as dietary fiber, fat and minerals that play a relevant role in the potential uses of winemaking residues in foodstuffs. Moreover, phenolic compounds and dietary fiber are considered the most important compound present in these residues, because of their beneficial effects on human health. For instance, it is well known that dietary fiber promotes beneficial physiological effects, including improvement of gastrointestinal function, moderation of postprandial insulin response, and reduction of total and low-density lipoprotein (LDL) cholesterol [82, 99, 100]. Moreover, dietary fiber consumption is related to the reduction of risk associated with cardiovascular diseases, diabetes prevention, cancer protection, and obesity prevention [79, 101]. Some fiber compounds in grape pomace are associated with phenolic substances and thus create antioxidant dietary fibers [100, 102, 103]. This gives the pomace stronger radical scavenging potential and higher nutritive value when compared with dietary fibers in cereals.

The polyphenols such as resveratrol, procyanidins/condensed tannins, anthocyanins, flavanols, and flavanols/catechins exert antioxidant properties [104]. Phenolic compounds from grape pomace are effective free radical scavengers and can potentially prevent cancer, cardiovascular diseases, cellular oxidative stress, diabetes, and pathogen proliferation. Thus, regarding the nutritional composition of winemaking by-products, it may be a source of different nutrients.

The main approaches in valorising winery by-products include the fortification of food. The incorporation of certain nutrients into food promote protection against oxidative processes, antimicrobial action, protection of probiotics, and natural coloring, among others. According to the literature, a rich content of dietary fiber and polyphenols present in the grape pomace has an advantage both from a nutritional and technological point of view and thus can be used as fortification element [83].

From a health benefit perspective, enriched diets with dietary fiber correlate with reduction of the glycemic response and plasma cholesterol and function as prebiotic, while phenolic compounds are well known for their antimicrobial and antioxidant effects. These compounds in the intestine tract exclude certain pathogenic bacteria while enhancing the growth of particular beneficial bacteria strain. *Leal et al.* (2020) showed that bioactive compounds extracted from white grape stems have antimicrobial activity against, essentially, Gram-positive bacteria, with efficacy in some cases higher than the commercial

antibiotics [105]. Moreover, the ability to control bacteria's growth can be successfully applied from a technological point of view as food preservation method. According to the research, the addition of grape pomace in products resulted in significantly increased oxidative stability, extended shelf life, and higher level of total phenolic content [75, 106]. Iuga *et al.* (2020) demonstrated that the powdered grape pomace when added to baked goods, increase their fiber and antioxidant content, and presenting the advantage of being gluten-free product [75]. However, the changes to reddish and bluish darken colours as a result of these fortifications in this case were considered as downside.

### 5.3 Skin Products/Cosmetics

Over the past years, the cosmetic industry has focused on developing new products that consist of formulations of natural origin or mainly natural ingredients. This emerges as a response to the consumers' concerns about allergies and possible health risks from long-term exposure to synthetic compounds, as well as growing awareness of environmental problems. In fact, natural ingredients have advantages over synthetic compounds since they exhibit functional and therapeutic properties beneficial to human beings and are biodegradable. Thus, the industry has focused on using bioactive compounds from agricultural by-products as it helps to develop a value-added product and is more appealing to consumers [80].

Grape polyphenols are widely used in cosmetic preparations due to their pronounced antioxidant effects. The presence of antioxidant compounds is essential to the preservation and performance of creams and other cosmetic products, as many ingredients present in the formulations are susceptible to suffering oxidation, and antioxidants prevent this [80, 107]. Therefore, these substances are not only used as a bioactive ingredient beneficial to the skin, protecting against free radicals, thus preventing skin aging, loss of elasticity, and wrinkles, but also to increase cosmetics' shelf life. Moreover, grape pomace and especially grape seeds possess antibacterial and antifungal properties, which can be valuable in skin care cosmetics. Hence, the opportunity to take advantage of the winemaking by-product extracts arises since they can be incorporated into cosmetic products, thus increasing their commercial value. However, winery wastes should be treated before entering into cosmetic formulations, as heavy metals and pesticides may be present [33].

Several cosmetic products containing winery extracts are present in the market, including face peeling with *Vitis vinifera* seed oil, sunscreens, anti-aging creams, scrubs, and day or night creams. Namely, Caudalie, one of the main skincare companies, is known to use grape and grapevine extracts in many of their products.

## **Chapter II**

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### **Objectives**

## **1. Main objectives**

Considering the facts, it is important to add value to the agro-food industry by-products. The incorporation of these by-products into the food chain was the main motivation for this work.

The main objective of this dissertation was the nutritional and antioxidant characterization of the major winemaking industry wastes, such as grape stalks and pomace.

Nutritional characterization consisted of the determination of the following:

- Moisture
- Total ash
- Total fat content
- Total, soluble and insoluble dietary fibre content
- Total protein content, protein nitrogen and non-protein nitrogen
- Remaining carbohydrates

Antioxidant characterization consisted in:

- Total phenolic content (TPC)
- Total flavonoid content (TFC)
- Ferric reducing antioxidant power (FRAP) assay
- DPPH• scavenging assay

Subsequently, this dissertation discusses the possible incorporation strategies of these by-products.

## **Chapter III**

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### **Material and Methods**

## 1. Chemicals and Reagents

Reagents and chemicals were of analytical grade:

- Total protein content and non-protein nitrogen: Trichloroacetic acid, Kjeldahl tablets, sulfuric acid ( $H_2SO_4$ ), sodium hydroxide (NaOH) from Merck (Darmstadt, Germany), and boric acid 4% ( $H_3BO_3$ ) from Panreac (Barcelona, Spain).
- Total fat content: Anhydrous sodium sulphate ( $Na_2SO_4$ ) was acquired in Merck (Darmstadt, Germany), sand in VWR Chemicals (Alfragide, Portugal), and petroleum ether in Carlo Erba Reagents (Val de Reuil, France).
- Total and insoluble dietary fibre content: fibre enzymes kit and celite were purchased in Sigma-Aldrich (St. Louis, USA), acetone in Fischer Chemical (U.K), ethanol 96% in AGA (Prior Velho, Portugal), chloridric acid in Carlo Erba (Val de Reuil, France), and sodium dihydrogen phosphate and di-sodium hydrogen phosphate anhydrous in Merck (Darmstadt, Germany).
- TPC: Folin-Ciocalteu reagent and gallic acid were purchased in Sigma-Aldrich (St. Louis, USA), and sodium carbonate ( $Na_2CO_3$ ) in Merck (Darmstadt, Germany).
- TFC: Catechin and aluminium chloride ( $AlCl_3$ ) and sodium nitrite ( $NaNO_2$ ) were purchased in Sigma-Aldrich (St. Louis, USA), absolute ethanol in Merck (Darmstadt, Germany), and sodium hydroxide (NaOH) from Merck (Darmstadt, Germany).
- FRAP assay: TPTZ (2,4,6-tripyridyltriazine), ferric chloride ( $FeCl_3$ ), heptahydrate ferrous sulphate, and sodium acetate were purchased in Sigma-Aldrich (St. Louis, USA).
- DPPH radical-scavenging activity assay: DPPH• (2,2-diphenyl-1-picrylhydrazyl radical) and trolox were purchased in Sigma-Aldrich (St. Louis, USA), and absolute ethanol in Merck (Darmstadt, Germany).

A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to obtain ultra-pure water.

## 2. Samples

The studied samples, by-products of the winemaking process, provided by three different Portuguese wineries (Herdade do Peso, Quinta do Centro, and Quinta do Sairrão) were stem and pomace (without any separation in their components).

Stems were directly obtained after the grape-destemming process at the beginning of the winemaking process and the whole pomace after pressing the grape and maceration for

around 8 days. Sample codifications, varieties, and corresponding regions are described in Tables 4 and 5.

**Table 4:** Grape pomace samples used in the study.

Region	Winery	Variety	Sample codification
<b>Alentejo</b>	Quinta do Centro	TN	BA1
		AB	BA2
		TN	BA3
	Herdade do Peso	Aragonez	BA4
		TN	BA5
<b>Douro</b>	Quinta do Sairrão	57%TN+ 38%TF+ 2%Tinta Francisca+ 3%Sousão	BA6
		TF	BA7
		68%TF + 32%TN	BA8

TN – Touriga Nacional; AB – Alicante Bouschet; TF – Touriga Franca

**Table 5:** Grape stem samples used in this study.

Region	Winery	Variety	Sample codification
<b>Alentejo</b>	Quinta do Centro	TN	E1
		Syrah	E2
		AB	E3
	Herdade do Peso	TN	E4
		AB	E5
<b>Douro</b>	Quinta do Sairrão	AB	E6
		TN	E7
		Arinto	E8

TN – Touriga Nacional; AB – Alicante Bouschet

## 2.1 Samples preparation

Stem samples were dried naturally in the sun (Figure 10). Pomace samples were stored at -20 °C. Prior to the analysis, samples were freeze-dried (Telstar Cryodos-80 Terrassa, Barcelona) at -76 °C, 0.032 mBar for 48h; milled (Figure 11) and stored in vacuum bags at room temperature until use.



**Figure 10:** Grape stem (left) and grape pomace (right).



**Figure 11:** Milled grape pomace and stem samples

### **3. Nutritional analysis**

Ash, fat, protein (protein and non-protein nitrogen), and dietary fibre (total, soluble and insoluble) contents were accomplished according to AOAC methods [108]. An infrared balance was used to determine the moisture content (KERN DBS, Balingen, German). The results are presented in g/100 g, in dry weight (dw).

#### **3.1 Moisture**

The moisture content was determined in an Infrared balance (KERN DBS, Baligen, German) (Figure 12). In particular,  $\approx 5$  g of sample was uniformly distributed in aluminium dishes and dried at  $105\text{ }^{\circ}\text{C}$  until constant weight. Moisture results are presented in g/100 g.



All samples were weighted ( $\approx 5$  g), in triplicate, to a porcelain capsule and then mixed with anhydrous sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) and treated sand. Subsequently, this mixture was transferred to Soxhlet extraction cartridges and placed in Soxhlet extraction ampoules. The ampoules were then adapted to flat bottom balloons, previously dried, cooled, tared, and numbered.

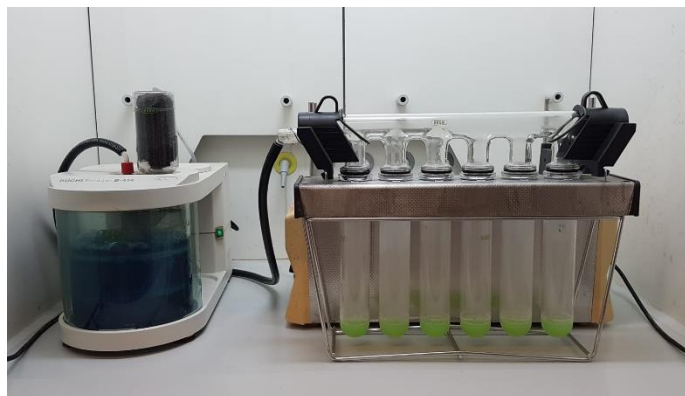
The extraction was carried out with petroleum ether in a Huber mini chiller and a heating mantle P Selecta (Figure 14) for 8 h. Afterwards, the petroleum ether was recuperated, and the flat bottom balloons were placed in an oven at  $105^\circ\text{C}$  for 1 h. Then, were weight and put in the oven for another 30 min at  $105^\circ\text{C}$ . This procedure was repeated until reaching the constant weight. The results are expressed in g/100 g dw.



**Figure 14:** Fat extraction by Soxhlet method.

### **3.4 Total nitrogen, protein nitrogen and non-protein nitrogen**

Total protein was determined according to an official method (AOAC 928.8). According to this method, the sample ( $\approx 1$  g) was weighted in triplicate to free-nitrogen vegetal paper, and placed in Kjeldahl tubes, together with 2 Kjeldahl tablets and 20 mL of sulfuric acid (96%). Protein digestion was performed for approximately 1 h, in an Automat K-438 equipment from Büchi Labortechnik AG (Flawil, Swiss), and the produced vapours were neutralized in a Scrubber B-414 (Figure 15).



**Figure 15:** Protein digestion.

Ammonia was distilled in a Distillation Unit K-360 (Büchi Labortechnik AG, Flawil, Swiss) and collected in a solution of 4% boric acid, which was then titrated with 0.2 M sulfuric acid, using methyl red as indicator (Figure 16).



**Figure 16:** Kjeldahl Distillation Unit and titration.

Non-protein nitrogen was determined using a similar protocol, but the sample was subjected to a previous precipitation step (Büchi Labortechnik AG, 2010) [109], with some modifications as described by Machado *et al.* (2020) [110].

Non-protein nitrogen content was calculated according to Büchi Labortechnik AG (2010) [109]. Protein nitrogen content was used to calculate real protein content using the conversion factor 6.25 [111]. Analyses were performed in triplicate, and the results are presented in g/100 g dw.

### 3.5 Total, soluble and insoluble dietary fibre

The total dietary fibre was determined according to AOAC 985.29 method by enzymatic digestion (Figure 17).

Samples were weighted ( $\approx 0.5$  g), in quadruplicate, to beakers. Then, 50 mL of phosphate buffer 0.08M (pH=6) and  $\alpha$ -amylase were added to samples to proceed with the first digestion step (95 °C, 20 min).

After cooling to room temperature, 10 mL of NaOH (0.275 M) was added. The pH was then adjusted to  $7.5 \pm 0.2$  so the protease (50 mg/mL) enzymatic activity could occur (60 °C, 35 min).

Then, after cooling to room temperature, 10 mL of HCl (0.325 M) was added. The pH was adjusted to 4 - 4.6 so that the amyloglucosidase enzymatic activity could take place (60 °C, 35 min).

Finally, after cooling to room temperature, 200 mL of ethanol (95%) was added to the beakers, and the solution was left during the night to form a precipitate.

The precipitates were filtrated in pre-weighted VELP glass crucibles and using celite as a filter. Filtration took place using 60 mL of ethanol (78%), 20 mL of ethanol (96%), and 20 mL of acetone.

Finally, the VELP glass crucibles containing the residues were put in an oven overnight, cooled, and weighted. Two residues were used for protein quantification. The other two residues were incinerated (525 °C for 5 h), cooled, and weighted.



**Figure 17:** Enzymatic digestion (left) and filtration (right).

The insoluble dietary fibre determination consists of the same protocol as total dietary fibre except for the precipitation during the night, which occurs without the addition of the 200 mL of ethanol (95%), and in the filtration, samples are washed using 20 mL of distilled water, 20 mL of ethanol (96%) and 20 mL of acetone.

### **3.6 Remaining Carbohydrates**

The remaining carbohydrates were determined by difference. Results are presented in g/100 g dw and were obtained using the following equation:

% Remaining carbohydrates = 100 – (% moisture + % protein + % fat + % ash + % total fiber)

## **4. Phytochemicals and antioxidant activity**

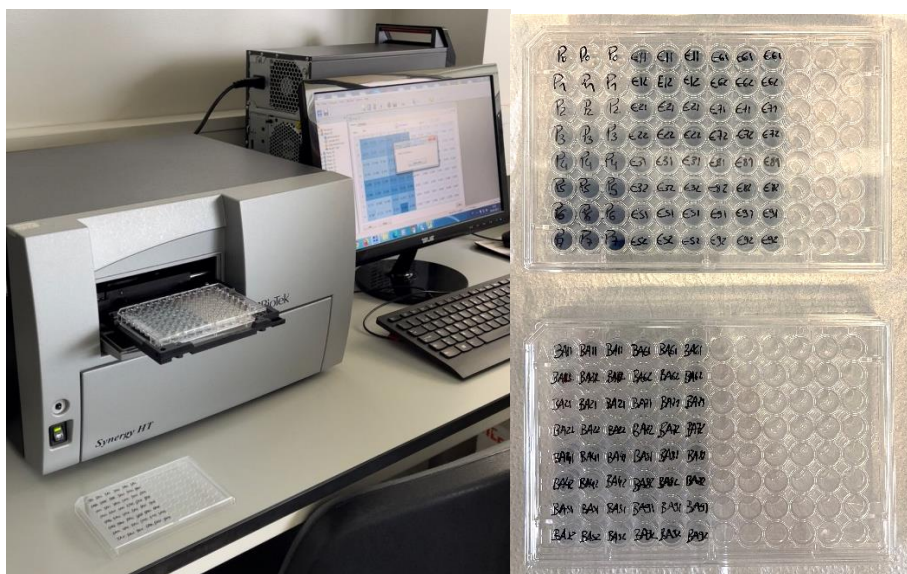
Phytochemical extraction was performed in triplicate, and according to the protocol described by Costa *et al.* (2014) [112] with slight modifications. Briefly, samples (100 mg) were mixed in 200 mL of absolute ethanol and deionized water (50/50 v/v) under constant stirring for 40 min at 40 °C. Later, extracts were filtered with a paper filter, and aliquots (900 µL) were stored in 1 mL Eppendorfs® at -20 °C for further analysis.

### **4.1 Total phenolics content (TPC)**

The amount of total phenolics was determined using the Folin-Ciocalteu method as described by Singleton *et al.* (1965) [113] with some modifications by Alves *et al.* (2010) [114].

In the microplate, 30 µL of extracts were mixed with 150 µL of Folin-Ciocalteu reagent (1:10) and 120 µL of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) (7.5%, m/v). The microplate was incubated at 45 °C for 15 minutes and then cooled down at room temperature for 30 minutes, protected from light.

A microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) (Figure 18) was used to determine the samples' absorbance (765 nm). Gallic acid was used for constructing the standard curve ( $y=0.0107x + 0.0161$ ;  $R^2=0.9986$ ) and the results were expressed as mg of Equivalents of gallic acid/ g of sample (mg GAE/ g).



**Figure 18:** Microplate reader (left) and microplate (right).

## 4.2 Total flavonoids content (TFC)

Flavonoid contents in the extracts were determined by a colorimetric method described by Costa *et al.* (2014) [112]. Aliquots of 1 mL of extract were mixed with 4 mL of distilled water and 300  $\mu$ L of NaNO<sub>2</sub> (5%). After 5 min, 300  $\mu$ L of AlCl<sub>3</sub> (10%), and 1 min after 2 mL sodium hydroxide (1 M) and 2.5 mL of distilled water were added. The solution was mixed well, and the absorbance was measured at 510 nm in a microplate (Figure 19) using a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA),

Total flavonoids content was calculated through a calibration curve of catechin ( $y=0.0031x+0.0026$ ;  $R^2=0.9999$ ) and expressed as mg of catechin equivalents (CE)/g of sample (mg CE/g).



**Figure 19:** Microplate with prepared samples and calibration curve.

### 4.3 Antioxidant activity

#### 4.3.1. Ferric-reducing antioxidant power (FRAP)

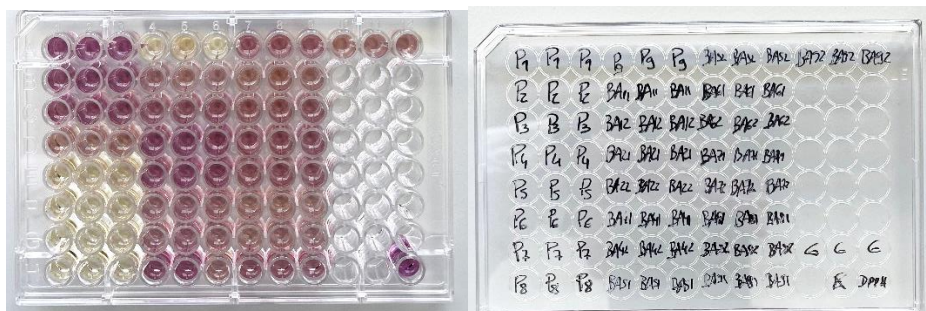
The FRAP assay was performed according to Benzie and Strain (1996) [115] with slight modifications. Briefly, in the microplate, 35  $\mu$ L of the extract was mixed with 265  $\mu$ L of FRAP reagent (containing 0.3 M acetate buffer, 10 mM TPTZ solution, and 20 mM of ferric chloride). Then, the microplate was kept for 30 min at 37 °C protected from light.

A microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) was used to determine the samples' absorbance at 595 nm. A calibration curve was prepared with ferrous sulphate (50 - 400 mg/L;  $y=0.0023x - 0.002$ ;  $R^2= 0.9999$ ), and ferric reducing antioxidant power was expressed as mg of ferrous sulphate equivalents (FSE)/g of extract.

#### 4.3.2. DPPH radical-scavenging activity

The radical scavenging ability of extracts was analysed according to the method described by Costa *et al.* (2014) [112]. Briefly, in a microplate, 30  $\mu$ L of extract was mixed with 270  $\mu$ L of the DPPH• solution in ethanol ( $6 \times 10^{-5}$  M). A trolox calibration curve was prepared (5.62 - 175.34 mg/L;  $y=-0.0058x+0.5388$ ;  $R^2=0.9950$ ). A microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) was used to determine the samples' absorbance (525 nm) every 2 min for 40 min (Figure 20).

The results are presented in mg trolox equivalents/g (mg TE/g) of sample.



**Figure 20:** Microplate after reading DPPH• scavenging activity

### 4.4 Statistical analysis

All the assays were carried out in triplicate, data are expressed as mean  $\pm$  standard deviation. One-way ANOVA was used to reveal significant differences between samples, followed by Tukey post hoc test to make pairwise comparisons between means. Statistical significance of the difference between two groups was evaluated by Student's t-test. The level of significance for all hypothesis tests ( $p$ ) was 0.05. Statistical treatments were carried out using the IBM SPSS 26 (SPSS Inc., Chicago, IL, USA).

## **Chapter IV**

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### **Results and Discussion**

## 1. Nutritional profile

The composition of grapes may vary depending on viticultural practices, climatic conditions, soil, grape maturity, variety, and sanitary conditions [37]. Similarly, both the type of process and the conditions under which winemaking is carried out notably influence the composition of wine pomaces. Variability between grape varieties and the different effects of oenological practices on the composition of wine explains the variation reported in the literature on the composition of winemaking by-products.

The results of the chemical composition of winemaking by-products, namely grape stems and pomace are shown in Table 6. Both the grape stem and pomace samples showed an average of 8% for moisture values, although grape pomace samples varied more between them, with the lowest moisture value of 4.59% and the highest reaching 13.26%. The samples with the highest total protein content were grape pomace, ranging between 10.26 and 12.37%, in contrast with grape stalks samples, ranging from 3.28 to 5.27 %. The percentage of protein in grape pomace was similar to those reported by other authors, establishing between 6 and 15% (dry weight) [97, 101]. Similarly, Mora-Garrido et al. (2022) reported the percentage of protein ranging between 6 and 10% in Airén and Tempranillo varieties [116].

**Table 6:** Chemical composition of winemaking by-products in g/100 g dw.

Parameters	Grape Pomace	Grape Stalks
Moisture	4.59 – 13.26	7.15 – 9.75
Protein	10.26 – 12.37	3.28 – 5.27
Ash	4.68 – 7.62	5.86 – 9.35
Fat	6.66 – 10.50	0.32 – 1.19
Remaining Carbohydrates	72.48 – 75.34	84.86 – 89.89

As mentioned above, several studies described the high protein content of grape pomace, but its non-protein nitrogen content and complete amino acid profile are still unknown. The determination of complete amino acid composition could be of interest, especially if considering food supplement applications. In fact, in future works, the amino acid profile namely of grape pomace can be used to estimate its protein quality and its real protein content. As the first step to this goal, the non-protein nitrogen content of grape pomace and the grape stem was determined. According to the results, only 0.03 to 0.13% of the sample

nitrogen refers to the non-protein fraction, so only the protein nitrogen must be used to calculate the real protein content (Table 7).

The ash content of the grape pomace samples ranged from 4.68 to 7.62%. Similar results were reported by Jin et al. (2019), with ash content in grape pomace between 4 and 6% using eight commercial white and red pomace [117]. Regarding grape stalk samples, the ash content is on average, slightly higher, ranging between 5.86 and 9.35%. The fat content of the grape pomace samples was significantly higher (6.66 – 10.50%) than the amount found in the grape stalk samples (0.32 – 1.19%). This is due to the presence of grape seeds in the grape pomace samples, characterized by high lipid content [75]. In the same way, Jin et al. (2019) reported that the lipid contents of eight GPs ranged from 4.62% to 12.5% [117]. The lipid percentage of the grape stalk was lower than those described by Rivas et al. (2021), with 1.95% for the Tempranillo variety [118] but similar to those published by Llobera et al. (2007), that is 1.65% for Manto Negro variety.

**Table 7:** Total protein content and no-protein nitrogen expressed in g/100 g dw.

Sample	Region	Variety	Total protein	Non-protein nitrogen
BA4	Alentejo	Aragonez	11.91 ± 0.27	0.03
BA5		TN	12.26 ± 0.02	0.13
E7	Douro	TN	5,27 ± 0,25	0.04
E8		Arinto	4,77 ± 0,01	0.10

Results are expressed in g/100 g as the mean ± standard deviation in dry weight.

The remaining carbohydrate content is the main component of grape pomace and grape stalk, over 70% of the dry weight, which is in accordance with the values described in several studies [99, 101, 116, 117].

Considering the fact that grapes are well known as one of the most significant sources of polyphenols, mainly present in their seeds, skins, and short stems [40], the phytochemical (total phenols and flavonoids) and antioxidant activity (FRAP and DPPH•) of by-product were analysed. In fact, the presence of bioactive components like phenolic and flavonoid compounds is well described in the literature for the grape pomace, in contrast to the existing studies on the grape stem, considerably less.

The results presented in **Table 8** reveal that the grape stalk is richer in phenolic and flavonoid compounds than the grape pomace, and consequently, the antioxidant capacity is particularly higher than the values obtained for the grape pomace extracts. For instance, the

TPC for the grape stalk extracts ranges between 49.02 – 70.16 mg GAE/g dw, which is noticeably higher than the values found for the grape pomace extracts (21.96 – 48.68 mg GAE/g dw). Likewise, the TFC for the grape stem is roughly two times higher than for the pomace extracts. Other authors also reported a higher polyphenolic content in stems than in pomaces for different red grape varieties [99, 119, 120]. Indeed, Llobera et al. (2005) reported that the total extractable polyphenols for the grape stem of the Manto Negro variety is 116 mg GAE/g, considerably higher than the value found for the grape pomace of the same variety (26.3 mg GAE/g).

The grape stalk extracts showed higher antioxidant activity both by the DPPH• method (90.25 – 134.03 mg TE/g dw) and FRAP (646.45 – 1094.75 µmol FSE/g dw), when compared to the grape pomace extracts. These results agree with the total phenolic and flavonoid content observed in the samples. In fact, there was a high correlation between their antioxidant activity both by FRAP and DPPH• methods and their content of phenolic compounds. These compounds can act as metal chelators, hydrogen donors, reducing agents, and superoxide radical scavengers [80]. Their antioxidant capacity relates to the ability to reduce or inhibit free radicals. This correlation has been corroborated by previous studies [99, 118, 121, 122].

**Table 8:** Total phenolics and flavonoid contents and *in vitro* antioxidant activity (DPPH• inhibition and FRAP assays) of grape pomace and grape stalks samples.

Parameters	Grape Pomace	Grape Stalks
TPC (mg GAE/g dw)	21.96 – 48.68	49.02 – 70.16
TFC (mg CE/g dw)	14.85 – 34.92	27.76 – 46.93
FRAP (µmol FSE/g dw)	324.96 – 750.58	646.45 – 1094.75
DPPH• scavenging activity (mg TE/g dw)	37.52 – 88.35	90.25 – 134.03

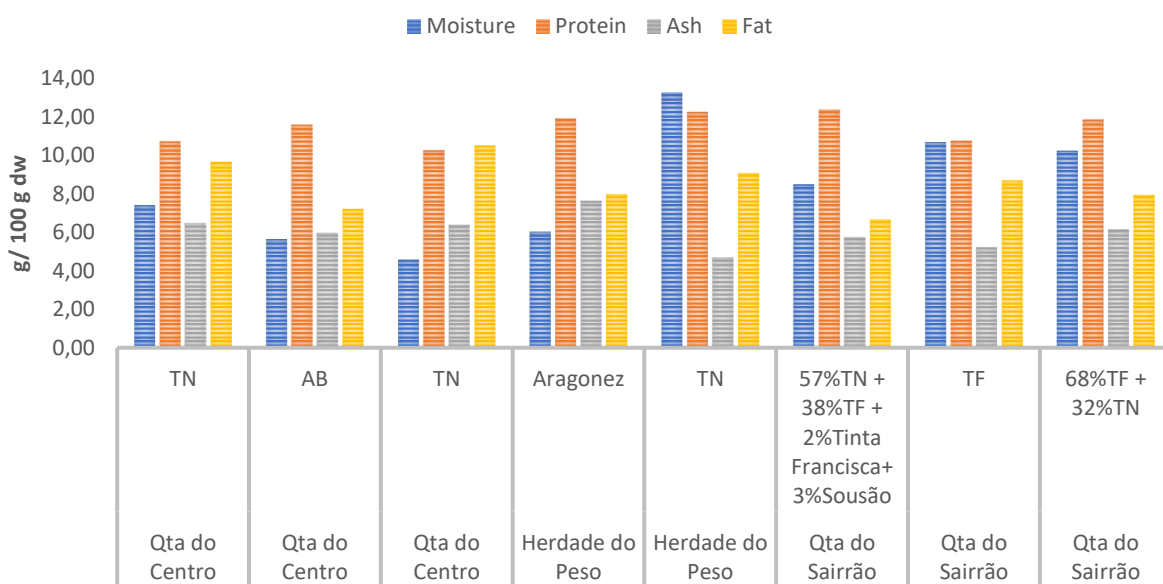
### 1.1 Grape pomace

Most grape production is used for winemaking, and grape pomace is one of the major solid by-products generated [123]. According to the Commission delegated regulation (EU) 2018/273, grape pomace means "the residue from the pressing of fresh grapes, whether or not fermented". Thus, grape pomace consists of fruit skins, seeds, and in some instances, some stems left over after the crushing and pressing stages of wine production.

The amount of pomace generated, its chemical composition, and nutritional value strongly depends on the type of press used for pressing whole grapes, the variety and maturity of grapes, and the terroir [61]. The variation reported in the literature on the composition of wine pomace can be explained by different effects of each winemaking process on the composition of wine and variability between grape varieties [79].

The chemical composition of grape pomace samples used for this study, collected in different regions and from various varieties, is presented in Table 10. Overall, grape pomace samples had a moisture content of 4.59 to 13.26%, corresponding to the samples of TN from Quinta do Centro and Herdade do Peso, respectively. Results showed that all grape pomace varieties registered similar protein and remaining carbohydrate content ( $p > 0.05$ ), with no significant differences between them.

Regarding the ash content, Aragonez was significantly rich in ash (7.62%) compared to other varieties. Sample BA6, which corresponds to the known mixture of varieties, as described in Table 10, was significantly ( $p < 0.05$ ) low in total fat content (6.66%), while TN from Quinta do Centro (BA3) exhibited significantly ( $p < 0.05$ ) higher content (10.50%).



**Figure 21:** The nutritional composition (g/100 g dw) according to the winery and the variety of the grape pomace samples.

Regarding the TN variety, the samples were obtained from different wineries from Alentejo region, with a distance between wineries of 160 km. No significant difference ( $p < 0.05$ ) between the TN grape pomace collected on different days from Quinta do Centro was observed. However, the TN sample from Herdade do Peso was significantly rich ( $p < 0.05$ ) in moisture and low in ash when compared to TN grape pomace of Quinta do Centro. These differences may be explained by the different oenological practices applied in these wineries

and specific microclimates. Moreover, no particular pattern was found when comparing different varieties or wineries (Figure 21).

According to the literature, dietary fibre is the main component of the pomace, ranging from 50 to 75 g/100 g of dry weight [50, 75, 99]. DF has been recognized for having health benefits, it is well known that DF promotes beneficial physiological effects, including the reduction of risk associated with cardiovascular diseases, constipation, cancer protection, diabetes prevention, cholesterol reduction, and obesity prevention [82, 99, 100]. Indeed, according to international guidelines, dietary fiber daily intake is 25 – 35 g for adults [124].

In this work, only two grape pomace samples were chosen to determine TDF, IDF, and SDF, namely because of the large number of samples and the time required for the analysis. IDF has been described as the predominant dietary fiber fraction for the grape pomace. Indeed, the IDF content from the samples ranged from 53.23 to 58.84 g/100 g dw., and the content of SDF was between 2.24 and 2.94 g/100 g dw. These values agreed with those published by the other authors [75, 82, 118]. According to the literature, the insoluble fraction is mainly composed of cellulose, hemicellulose, and lignin, while soluble pectins are characteristic of soluble dietary fraction [125]. Acid-insoluble lignin (Klason lignin) is dietary fiber's main component in red and white wine pomace [37]. Cellulose is characterized by having multiple applications, and it is generally used in many areas, such as paper, food, and pharmaceutical industries. Grape pomace is considered a good source of DF, as it has an appropriate ratio of SDF and IDF and is known as an antioxidant dietary fiber [75]. Indeed, grape pomace could have enhanced preference over other sources of DF due to the presence of associated bioactive (phenolic compounds) with antioxidant properties, which impart additional health benefits [100, 102, 103].

**Table 9:** Total, soluble, and insoluble dietary fiber expressed in g/100 g dw.

Sample	Region	Variety	TDF	IDF	SDF	Remaining Carbohydrates
BA1	Alentejo	TN	56.78 ± 0.70	53.84 ± 0.83	2.94 ± 0.14	19.84 ± 0.59
BA2		AB	60.48 ± 0.11	58.23 ± 1.01	2.24 ± 0.90	16.64 ± 1.65

Results are expressed in g/100 g as the mean ± standard deviation in dry weight.

**Table 10:** Nutritional composition of grape pomace in g/100 g dw.

Sample	Region	Variety	Moisture	Protein	Ash	Fat	Remaining Carbohydrates
BA1		TN	7.42 ± 0.26 <sup>d</sup>	10.72 ± 0.32 <sup>a</sup>	6.45 ± 0.13 <sup>b</sup>	9.66 ± 0.02 <sup>b</sup>	73.16 ± 0.48 <sup>abc</sup>
BA2		AB	5.65 ± 0.03 <sup>e</sup>	11.58 ± 0.40 <sup>a</sup>	5.96 ± 0.19 <sup>cd</sup>	7.21 ± 0.11 <sup>e</sup>	75.25 ± 0.71 <sup>ab</sup>
BA3	Alentejo	TN	4.59 ± 0.12 <sup>f</sup>	10.26 ± 0.25 <sup>a</sup>	6.38 ± 0.08 <sup>bc</sup>	10.50 ± 0.02 <sup>a</sup>	72.86 ± 0.16 <sup>bc</sup>
BA4		Aragonez	6.03 ± 0.46 <sup>e</sup>	11.91 ± 0.27 <sup>a</sup>	7.62 ± 0.03 <sup>a</sup>	7.98 ± 0.28 <sup>d</sup>	72.48 ± 0.04 <sup>c</sup>
BA5		TN	13.26 ± 0.41 <sup>a</sup>	12.26 ± 0.02 <sup>a</sup>	4.68 ± 0.14 <sup>f</sup>	9.08 ± 0.25 <sup>bc</sup>	73.99 ± 0.42 <sup>abc</sup>
BA6	Douro	57% TN + 38%TF+ 2%Tinta Francisca+ 3%Sousão	8.49 ± 0.40 <sup>c</sup>	12.37 ± 0.02 <sup>a</sup>	5.72 ± 0.04 <sup>d</sup>	6.66 ± 0.13 <sup>f</sup>	75.25 ± 0.18 <sup>ab</sup>
BA7		TF	10.69 ± 0.37 <sup>b</sup>	10.75 ± 1.34 <sup>a</sup>	5.22 ± 0.08 <sup>e</sup>	8.69 ± 0.06 <sup>c</sup>	75.34 ± 1.36 <sup>a</sup>
BA8		68% TF + 32%TN	10.23 ± 0.08 <sup>b</sup>	11.85 ± 0.56 <sup>a</sup>	6.14 ± 0.01 <sup>bcd</sup>	7.94 ± 0.05 <sup>d</sup>	74.07 ± 0.50 <sup>abc</sup>

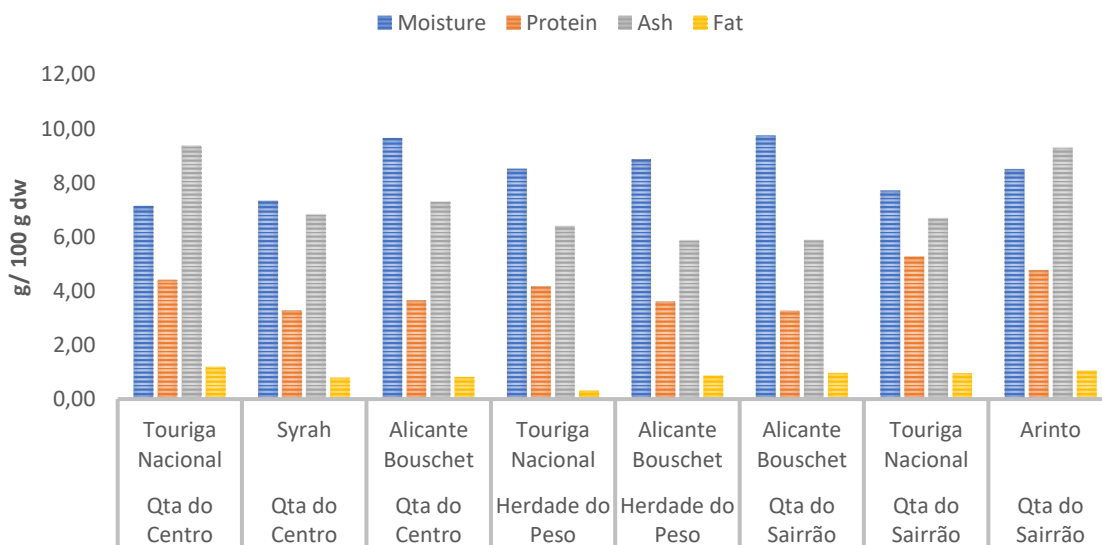
The results are presented as mean ± standard deviation. Within each column, different letters represent significant differences among individual samples at  $p < 0.05$ . For all parameters, no significant differences ( $p > 0.05$ ) were observed when Alentejo and Douro regions were compared.

## 1.2 Grape stem

Stalks of the white and red grapes (about 7% w/w of total grape weight) come from the destemming phase, constituting 2 to 12% of the wastes obtained in a winery. They are usually removed before fermentation to avoid excessive wine astringency [85, 87, 126].

The characterization of different fractions of grape stalks has limited literature reporting, and focus is mainly given to fiber components.

The general chemical composition of grape stalks from different varieties and regions is presented in Table 12. Overall, results showed that all grape stalk varieties registered similar moisture content ( $p > 0.05$ ), around 8%. The highest and lowest total protein content in grape stalks were found in TN from Quinta do Sairrão (5.27%) and Syrah from Quinta do Centro (3.29%), respectively. Regarding the ash content, TN from Quinta do Centro was significantly rich in ash (9.35%) compared to other varieties. There was no significant ( $p > 0.05$ ) difference between all the samples regarding the total fat content, excluding the TN from Herdade do Peso, which exhibited significantly ( $p < 0.05$ ) lower fat content (0.32%).



**Figure 22:** The nutritional composition (g/100 g dw) according to the winery and the variety of the grape stalk samples.

The samples of AB and TN grape stalk varieties were obtained from different wineries in Douro and Alentejo regions. The comparison between the same varieties from different wineries, as well as between varieties, showed that there was no significant difference ( $p < 0.05$ ) in terms of the nutritional composition. Moreover, no particular pattern was found when comparing different grape stalk samples (Figure 22).

Compared to the grape pomace by-product, there are relatively few published studies regarding the chemical composition of grape stems and the differences between varieties.

The few published studies focus on phenolic compounds, antioxidant activity, and dietary fibers. Table 11 shows that the major constituent of TDF is IDF, more than 40%, whereas SDF ranges between 4.40 and 9.79%. Only two samples were characterized in terms of dietary fiber, in future works, it would be interesting to discriminate different components of grape stalk fiber, in order to explore possible novel applications of this by-product.

**Table 11:** Total, soluble, and insoluble dietary fiber expressed in g/100 g dw.

Sample	Region	Variety	TDF	IDF	SDF	Remaining Carbohydrates
E1	Alentejo	TN	51.92 ± 0.05	42.13 ± 0.83	9.79 ± 0.21	36.87 ± 0.25
E2		Syrah	52.07 ± 0.54	49.03 ± 0.15	4.40 ± 0.54	42.86 ± 0.53

Results are expressed in g/100 g as the mean ± standard deviation in dry weight.

**Table 12:** Nutritional composition of grape stem in g/100 g dw.

Sample	Region	Variety	Moisture	Protein	Ash	Fat	Remaining Carbohydrates
E1		TN	7,15 ± 0,40 <sup>c</sup>	4,41 ± 0,39 <sup>abc</sup>	9,35 ± 0,21 <sup>a</sup>	1,19 ± 0,05 <sup>a</sup>	85,06 ± 0,12 <sup>d</sup>
E2		Syrah	7,34 ± 0,56 <sup>bc</sup>	3,29 ± 0,13 <sup>c</sup>	6,82 ± 0,52 <sup>bc</sup>	0,79 ± 0,02 <sup>a</sup>	89,10 ± 0,36 <sup>ab</sup>
E3	Alentejo	AB	9,66 ± 0,47 <sup>ab</sup>	3,66 ± 0,54 <sup>bc</sup>	7,29 ± 0,44 <sup>b</sup>	0,81 ± 0,07 <sup>a</sup>	88,24 ± 0,03 <sup>bc</sup>
E4		TN	8,52 ± 0,68 <sup>b</sup>	4,17 ± 0,12 <sup>abc</sup>	6,39 ± 0,37 <sup>bc</sup>	0,32 ± 0,04 <sup>b</sup>	89,12 ± 0,29 <sup>ab</sup>
E5		AB	8,87 ± 0,25 <sup>b</sup>	3,62 ± 0,41 <sup>bc</sup>	5,86 ± 0,04 <sup>c</sup>	0,86 ± 0,08 <sup>a</sup>	89,66 ± 0,53 <sup>a</sup>
E6		AB	9,75 ± 0,79 <sup>ab</sup>	3,28 ± 0,27 <sup>c</sup>	5,87 ± 0,14 <sup>c</sup>	0,96 ± 0,19 <sup>a</sup>	89,89 ± 0,59 <sup>a</sup>
E7	Douro	TN	7,72 ± 0,20 <sup>bc</sup>	5,27 ± 0,25 <sup>a</sup>	6,67 ± 0,18 <sup>bc</sup>	0,95 ± 0,18 <sup>a</sup>	87,11 ± 0,25 <sup>c</sup>
E8		Arinto	8,51 ± 0,35 <sup>b</sup>	4,77 ± 0,01 <sup>ab</sup>	9,28 ± 0,05 <sup>a</sup>	1,04 ± 0,01 <sup>a</sup>	84,86 ± 0,01 <sup>d</sup>

The results are presented as mean ± standard deviation. Within each column, different letters represent significant differences among individual samples at  $p < 0.05$ . For all parameters, no significant differences ( $p > 0.05$ ) were observed when Alentejo and Douro regions were compared.

## 2. Bioactive compounds and antioxidant activity

Phenolic compounds are nowadays a highly investigated class of bioactive compounds [127]. They include various families of compounds that are distributed widely in nature and mainly found in vegetable-derived foods. In addition, phenolic compounds interact with the salivary glycoproteins, thus being responsible for the fruit bitterness. Also, phenolics are known to account for the colour of many fruits and vegetables and the differences in the flavour and colour of different wines.

Chemically, phenolic compounds contain one or more aromatic rings that are linked to one or more hydroxyl groups [127]. Furthermore, according to their basic chemical structure, they may be divided into different groups: phenolic acids, flavonoids, tannins, stilbenes, and lignans [127, 128]. These compounds are considered powerful antioxidants; some even have antimicrobial or anticarcinogenic properties. Their capacity to donate an electron and/or a hydrogen atom to free radicals is why they are considered antioxidants [127]. Additionally, the presence of a high number of hydroxyl groups makes them toxic to microorganisms.

A popular technique to evaluate the amount of phenolic compounds is the Folin-Ciocalteu assay. This methodology is based on the phenolics' capacity to reduce the Folin-Ciocalteu reagent in alkaline conditions. A calibration curve is also prepared using a pure standard, mainly gallic acid. Regarding the determination of flavonoid content, a colorimetric based on the formation of a flavonoid-aluminium compound is used [129].

Currently, there are several methods for evaluating total antioxidant activity in foodstuffs, each with its advantages and limitations. Because of that, it is advisable to use more than one method to study the antioxidant profile. In this work, the extracts' antioxidant activity was assessed by two chemical-based methods: FRAP and DPPH• scavenging. FRAP assay evaluates the reduction of a ferric ion-ligan complex by an antioxidant to a blue-colored ferrous complex, considered an electron transfer reaction assay [130]. This reaction allows the detection of compounds with redox potential inferior to 0.7 V, being a reasonable screen for maintaining redox status in cells or tissues [112]. In turn, DPPH• scavenging activity estimates the antiradical activity of several chemicals based on the DPPH• neutralization by donating an electron or radical quenching via hydrogen transfer [112, 131].

Numerous works report the TPC, TFC, and antioxidant capacity, mainly of grape pomace extracts. There are fewer studies published regarding grape stems. Nevertheless, many of the works published used different extraction methods and solvents than the ones used in this work, which compromises the comparison of the results among studies. However, some

studies, as discussed below, demonstrate these ingredients' interesting TPC and antioxidant activities.

## 2.1 Grape pomace

The results of the TPC, TFC, and antioxidant activity considering FRAP and DPPH• scavenging activity are shown in Table 13. For all the analysed parameters, there are significant differences among the varieties.

The TPC varied between  $22.2 \pm 1.09$  (TF) and  $48.81 \pm 0.55$  (AB) mg GAE/g dw. When comparing these results with those described in Ky *et al.* (2014), which characterized six different French grape varieties, including AB and Syrah, with  $44.5 \pm 0.4$  and  $35.6 \pm 1.8$  mg GAE/g dw, respectively, the TPC value obtained in the present studies is higher [132]. Also, Ahmed *et al.* (2020) studied 10 different grape pomace varieties from Turkey, obtaining significantly lower results [122]. However, Tournour *et al.* (2017) have found TPC of  $142 \pm 1$  and  $135 \pm 4$  mg GAE/g for 2012 and 2013 vintages, respectively, for TN variety grape pomace [133]. These results are significantly higher when compared with the results of the present work.

Regarding TFC, values ranged between  $14.92 \pm 2.59$  (TF) and  $34.11 \pm 3.17$  (AB) mg CE/g dw. The results of the present study were significantly higher than those reported by Ghendov-Mosanu *et al.* (2022), who determined the TFC of grape pomace extracts by testing the influence of temperature and ethanol concentration to optimize the extraction technique [134].

TF exhibited higher TPC and TFC with a concomitant higher antioxidant activity whereas AB exhibited lower levels with low antioxidant activity than the other varieties. The significantly ( $p < 0.05$ ) higher total phenolics observed in TF variety leads to significantly ( $p < 0.05$ ) higher antioxidant activity of the variety compared to others. This observation is corroborated by different studies in which a linear relationship between total phenolics and antioxidant values was observed [81, 122, 132]. Regarding all parameters, no significant differences ( $p > 0.05$ ) were observed when Alentejo and Douro regions were compared.

The use of natural food preservatives and cosmetic applications is a trend followed by both food manufacturers and consumers [106]. Growing demand for a minimally processed product containing only natural additives will increase the necessity for developing natural alternatives. This is particularly interesting as many phenolic compounds exhibit significant antibacterial activity [90, 106, 135]. For instance, phenolic compounds from grape pomace are very effective against the specific virulence traits of *Streptococcus mutans*, despite major differences in their phenolic content [136]. Although the mechanism of these compounds' antibacterial action is unknown, several authors describe that its action

involves many sites at the cellular level [106, 134]. For example, flavonoids promote the formation of complexes and bind to the cell walls of bacteria, though they affect intracellular changes in pH, inhibit energy metabolism, and interfere with ATP and DNA synthesis [136, 137]. On the other hand, from the cosmetic point of view, grape pomace polyphenol exhibited promising antioxidant, antiaging, anti-hyperpigmentation, and UV-protecting activities with in vitro testing [138].

## 2.2 Grape stem

The results of total phenolics and antioxidant activity of analysed grape stem samples are shown in Table 14. The TPC varied between  $52.28 \pm 3.59$  (AB) and  $70.16 \pm 6.09$  (Syrah) mg GAE/g dw. When comparing these results with those described in the literature, it was found that obtained values in this work were generally lower. For instance, Leal *et al.* (2020) presented values ranging between  $94.71 \pm 4.65$  (Rabigato) and  $123.09 \pm 5.02$  (Malvasia Fina) mg GAE/g dw [105], whereas Esparza *et al.* (2021) showed values ranging  $82 \pm 1$  and  $172 \pm 6$  mg GAE/g dw [139]. TFC varied from  $27.76 \pm 3.24$  (AB) to  $46.93 \pm 8.43$  (Syrah) mg CE/ g dw.

In general, varieties differed significantly ( $p < 0.05$ ) concerning both the phenolic composition and antioxidant capacity of their stems. Furthermore, significant differences ( $p < 0.05$ ) were observed when stems from Alentejo and Douro were considered separately. Overall, the stem samples from the Alentejo region showed higher phenolic and flavonoid content and higher antioxidant capacity.

Moreover, Leal *et al.* (2020) evaluated the antioxidant capacity of the grape stem from different Portuguese varieties (Tinta Roriz, Touriga Nacional, Castelão, Syrah, Arinto, and Fernão Pires) and observed significant differences among varieties from the same vintage (2018) [107]. When comparing the results obtained in the present study with those obtained by Leal *et al.* (2020), we observe that they found antioxidant capacity values between  $0.35 \pm 0.00$  and  $0.84 \pm 0.06$  mmol Trolox/g in their samples, and these values are similar to those obtained in the present work. The same can be observed in the study by Esparza *et al.* (2021) which evaluated grape stem extracts obtained from different grape varieties from two vintages (2016 and 2018) [139].

The variations found on the phenolic composition in this work and in other studies found in the literature can be explained by the specific characteristics of each variety, the viticultural practices, and climate factors, among others [81, 105]. Indeed, the growing conditions significantly affect plants' phenolic composition. Gouvinhas *et al.* (2020) revealed that geographical region greatly impacts this parameter. This work demonstrated

that the samples of the grape stem in lower altitude sites (Lower Corgo sub-region) had a higher content of total phenols and flavonoids. Moreover, Esparza et al. (2021) observed that grape stems from the 2018 vintage presented higher TPC and TFC values when compared to the samples from the 2016 vintage (distinguished by extreme temperatures and accumulated precipitation). So, this study stresses the influence of environmental factors on the synthesis of polyphenols.

**Table 13:** Total phenolics and antioxidant activity of grape pomace samples.

Sample	Region	Variety	TPC	TFC	FRAP	DPPH
			mg GAE/g dw	mg CE/g dw	μmol FSE/g dw	mg TE/g dw
BA1	Alentejo	TN	32.89 ± 0.87 <sup>c</sup>	24.41 ± 3.18 <sup>c</sup>	483.88 ± 8.03 <sup>c</sup>	59.32 ± 3.63 <sup>bed</sup>
BA2		AB	48.81 ± 0.55 <sup>a</sup>	34.11 ± 3.17 <sup>a</sup>	715.82 ± 12.81 <sup>a</sup>	90.08 ± 5.01 <sup>a</sup>
BA3		TN	34.77 ± 1.29 <sup>c</sup>	25.92 ± 2.59 <sup>bc</sup>	525.28 ± 21.83 <sup>bc</sup>	66.11 ± 4.68 <sup>bc</sup>
BA4		Aragonez	35.17 ± 2.86 <sup>c</sup>	24.36 ± 0.82 <sup>c</sup>	565.32 ± 52.09 <sup>b</sup>	70.84 ± 11.38 <sup>abc</sup>
BA5		TN	21.52 ± 1.01 <sup>d</sup>	15.68 ± 1.59 <sup>d</sup>	317.43 ± 22.01 <sup>d</sup>	36.54 ± 4.37 <sup>d</sup>
BA6	Douro	57% TN + 38%TF+ 2%Tinta Francisca+ 3%Sousão	45.20 ± 0.52 <sup>b</sup>	28.86 ± 0.14 <sup>bc</sup>	659.85 ± 29.72 <sup>a</sup>	79.38 ± 2.39 <sup>ab</sup>
BA7		TF	22.2 ± 1.09 <sup>d</sup>	14.92 ± 2.59 <sup>d</sup>	348.06 ± 12.12 <sup>d</sup>	41.59 ± 3.32 <sup>cd</sup>
BA8		68% TF + 32%TN	47.11 ± 2.21 <sup>ab</sup>	30.45 ± 0.36 <sup>ab</sup>	698.69 ± 56.72 <sup>a</sup>	79.88 ± 10.06 <sup>ab</sup>

The results are presented as mean ± standard deviation. Within each column, different letters represent significant differences among individual samples at p<0.05. For all parameters, no significant differences

**Table 14:** Total phenolics and antioxidant activity of grape stem samples.

Sample	Region	Variety	TPC	TFC	FRAP	DPPH
			mg GAE/g dw	mg CE/g dw	μmol FSE/g dw	mg TE/g dw
E1	Alentejo	TN	63.89 ± 2.13 <sup>ab</sup>	43.25 ± 0.54 <sup>ab</sup>	1009.86 ± 26.97 <sup>ab</sup>	125.27 ± 6.96 <sup>ab</sup>
E2		Syrah	70.16 ± 6.09 <sup>a</sup>	46.93 ± 8.43 <sup>a</sup>	1094.75 ± 85.35 <sup>a</sup>	134.03 ± 5.50 <sup>a</sup>
E3		AB	52.28 ± 3.59 <sup>c</sup>	34.93 ± 1.25 <sup>cd</sup>	814.10 ± 83.64 <sup>c</sup>	104.07 ± 7.88 <sup>d</sup>
E4		TN	52.56 ± 2.30 <sup>c</sup>	40.10 ± 4.71 <sup>abc</sup>	798.18 ± 32.17 <sup>c</sup>	103.60 ± 4.20 <sup>d</sup>
E5		AB	63.94 ± 2.56 <sup>ab</sup>	44.28 ± 1.00 <sup>ab</sup>	1008.80 ± 31.63 <sup>ab</sup>	123.57 ± 6.46 <sup>ab</sup>
E6	Douro	AB	49.02 ± 5.98 <sup>c</sup>	27.76 ± 3.24 <sup>d</sup>	646.45 ± 80.04 <sup>d</sup>	90.25 ± 3.18 <sup>e</sup>
E7		TN	61.16 ± 2.88 <sup>b</sup> *	45.18 ± 5.49 <sup>a</sup> *	955.56 ± 35.64 <sup>b</sup> *	119.25 ± 8.45 <sup>bc</sup> *
E8		Arinto	53.48 ± 1.65 <sup>c</sup>	36.97 ± 2.14 <sup>bc</sup>	842.35 ± 30.13 <sup>c</sup>	108.66 ± 4.51 <sup>cd</sup>

The results are presented as mean ± standard deviation. Within each column, different letters represent significant differences among individual samples at p<0.05. \*, significant differences (p>0.05) among Alentejo and Douro regions.

## **Chapter V**

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### **Conclusion**

This dissertation's main goal consisted in evaluating wine industry by-products, namely grape pomace and stem, and their nutritional and phenolic composition, and antioxidant activity. The chemical composition and evaluation of the bioactive compound of these by-products are crucial to encourage and expand the areas of their eventual valorisation. As outlined previously, winery by-products' chemical composition is somewhat complex and varies according to numerous factors, such as terroir, grape variety, environmental factors, processing levels, the technology used, among others. Nevertheless, it was observed that grape pomace is composed mainly of remaining carbohydrates (on average 74 g/100 g dw). Protein, fat, and ash were also presented in minor quantities, on average 11.46, 8.47, and 6.02 g/100 g dw, respectively.

Additionally, it was corroborated that grape pomace is an important source of DF around 58 g/100 g dw, although only two samples were analysed. Further studies are needed in order to identify the dietary fiber present and, consequently, its possible application. Moreover, when comparing samples between different varieties and between regions no significant ( $p < 0.05$ ) differences were observed.

Regarding grape stems, very few studies are available in the literature describing their chemical composition. The remaining carbohydrate constitutes the main fraction of grape stem nutritional composition, with roughly 88 g/ 100g dw. Moreover, it is an important source of IDF, although further studies are needed. Protein, ash, and fat constitute a minor fraction of grape stem chemical composition with 4.06, 7.19, and 0.86 g/100 g dw, respectively.

Grape pomace and, to a lesser extent, grape stem are well known for their high phenolic content and antioxidant activity. This work not only reinforces the literature but also shows that grape stem has twice as much total phenolic and flavonoid content compared to grape pomace and, consequently, higher antioxidant activity. Further studies should focus on the characterization of these phenolic compounds and the study of green and profitable extraction techniques.

To conclude, the potential of winery by-products was proven once again in this dissertation. Additional studies are needed to characterize these residues in a more fundamental level, enabling an increase in the spectrum of their applications. The next stages should focus on scaling up studies and cost evaluation of the extraction techniques. Nevertheless, this dissertation allowed us to take one more step towards the potential valorisation and applications of winery by-products in several industries, including cosmetics, food, and pharmaceutical sectors, being possible to develop new sustainable and value-added products.

## References

1. OIV, *State of the world vitivinicultural sector in 2019*. International Organisation of Vine and Wine.
2. *Global Economic Prospects, June 2022*. Global Economic Prospects, June 2022. 2022, International Bank for Reconstruction and Development.
3. *World Population Prospects 2022: Summary of Results*. 2022, United Nations Department of Economic and Social Affairs, Population Division.
4. FAO, I., UNICEF, WFP and WHO, *In Brief to The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets*. FAO, 2020.
5. FAO, I., UNICEF, WFP and WHO, *The State of Food Security and Nutrition in the World 2022. Repurposing food and agricultural policies to make healthy diets more affordable*. 2022: Rome, FAO.
6. United Nations, D., Population Division, *World Population Prospects 2022*.
7. Dillard, H.R., *Global food and nutrition security: from challenges to solutions*. Food Security, 2019. **11**(1): p. 249-252.
8. Godfray, H.C.J., et al., *Food security: The challenge of feeding 9 billion people*. Science, 2010. **327**(5967): p. 812-818.
9. Tilman, D., et al., *Agricultural sustainability and intensive production practices*. Nature, 2002. **418**(6898): p. 671-677.
10. Stocker, T.F., et al., *Climate change 2013 the physical science basis: Working Group I contribution to the fifth assessment report of the intergovernmental panel on climate change*. Climate Change 2013 the Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Vol. 9781107057999. 2013. 1-1535.
11. Misiou, O. and K. Koutsoumanis, *Climate change and its implications for food safety and spoilage*. Trends in Food Science and Technology, 2022. **126**: p. 142-152.
12. IPCC, *Climate Change 2022: Mitigation of Climate Change. Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change 2022*, Cambridge, UK and New York, NY, USA: Cambridge University Press.
13. Friedlingstein, P., et al., *Global Carbon Budget 2021*. Earth System Science Data, 2022. **14**(4): p. 1917-2005.
14. Rocha, J., et al., *Impacts of climate change on reservoir water availability, quality and irrigation needs in a water scarce Mediterranean region (southern Portugal)*. Science of The Total Environment, 2020. **736**: p. 139477.
15. Yang, C., et al., *Assessment of irrigated maize yield response to climate change scenarios in Portugal*. Agricultural Water Management, 2017. **184**: p. 178-190.
16. Wheeler, T. and J. von Braun, *Climate Change Impacts on Global Food Security*. Science, 2013. **341**(6145): p. 508-513.
17. Iizumi, T., et al., *Crop production losses associated with anthropogenic climate change for 1981–2010 compared with preindustrial levels*. International Journal of Climatology, 2018. **38**(14): p. 5405-5417.
18. Farcas, A.C., et al., *Food Security during the Pandemic and the Importance of the Bioeconomy in the New Era*. Sustainability, 2021. **13**(1): p. 150.
19. Galanakis, C.M., *The food systems in the era of the coronavirus (CoVID-19) pandemic crisis*. Foods, 2020. **9**(4).
20. Singh, S., et al., *Impact of COVID-19 on logistics systems and disruptions in food supply chain*. International Journal of Production Research, 2021. **59**(7): p. 1993-2008.
21. Hobbs, J.E., *Food supply chains during the COVID-19 pandemic*. Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie, 2020. **68**(2): p. 171-176.

22. Pereira, M. and A.M. Oliveira, *Poverty and food insecurity may increase as the threat of COVID-19 spreads*. Public Health Nutrition, 2020. **23**(17): p. 3236-3240.
23. Zhou, X.Y., et al., *Influence of Russia-Ukraine War on the Global Energy and Food Security*. Resources, Conservation and Recycling, 2023. **188**.
24. Ben Hassen, T. and H. El Bilali, *Impacts of the Russia-Ukraine War on Global Food Security: Towards More Sustainable and Resilient Food Systems?* Foods, 2022. **11**(15).
25. Behnassi, M. and M. El Haiba, *Implications of the Russia-Ukraine war for global food security*. Nature Human Behaviour, 2022. **6**(6): p. 754-755.
26. Nasir, M.A., A.D. Nugroho, and Z. Lakner, *Impact of the Russian-Ukrainian Conflict on Global Food Crops*. Foods, 2022. **11**(19).
27. UN. *THE 17 GOALS | Sustainable Development 2022*. Available from: <https://sdgs.un.org/goals>.
28. Stübler, A.-S., V. Heinz, and K. Aganovic, *Development of food products*. Current Opinion in Green and Sustainable Chemistry, 2020. **25**: p. 100356.
29. Gómez-García, R., et al., *Valorisation of food agro-industrial by-products: From the past to the present and perspectives*. J Environ Manage, 2021. **299**: p. 113571.
30. Faustino, M., et al., *Agro-Food Byproducts as a New Source of Natural Food Additives*. Molecules, 2019. **24**(6).
31. Mirabella, N., V. Castellani, and S. Sala, *Current options for the valorization of food manufacturing waste: A review*. Journal of Cleaner Production, 2014. **65**: p. 28-41.
32. Geissdoerfer, M., et al., *The Circular Economy – A new sustainability paradigm?* Journal of Cleaner Production, 2017. **143**: p. 757-768.
33. Kalli, E., et al., *Novel application and industrial exploitation of winery by-products*. Bioresources and Bioprocessing, 2018. **5**(1): p. 46.
34. Sorvari, J. and M. Wahlström, *Chapter 17 - Industrial By-products*, in *Handbook of Recycling*, E. Worrell and M.A. Reuter, Editors. 2014, Elsevier: Boston. p. 231-253.
35. Bordiga, M., *Valorization of Wine Making By-Products*. 2016: Taylor & Francis Group.
36. Hogervorst, J.C., U. Miljić, and V. Puškaš, *5 - Extraction of Bioactive Compounds from Grape Processing By-Products*, in *Handbook of Grape Processing By-Products*, C.M. Galanakis, Editor. 2017, Academic Press. p. 105-135.
37. García-Lomillo, J. and M.L. González-SanJosé, *Applications of Wine Pomace in the Food Industry: Approaches and Functions*. Comprehensive Reviews in Food Science and Food Safety, 2017. **16**(1): p. 3-22.
38. *Food and Agriculture Organization of the United Nations—Statistics Division*. FAOSTAT: <http://faostat3.fao.org/home/E>.
39. Bharathiraja, B., et al., *Critical review on bioconversion of winery wastes into value-added products*. Industrial Crops and Products, 2020. **158**: p. 112954.
40. Coelho, M.C., et al., *The use of emergent technologies to extract added value compounds from grape by-products*. Trends in Food Science & Technology, 2020. **106**: p. 182-197.
41. Mendes, J.A.S., et al., *Towards comprehensive utilization of winemaking residues: Characterization of grape skins from red grape pomaces of variety Touriga Nacional*. Industrial Crops and Products, 2013. **43**: p. 25-32.
42. Zhang, N., et al., *Sustainable options for the utilization of solid residues from wine production*. Waste Management, 2017. **60**: p. 173-183.
43. Maicas, S. and J.J. Mateo, *Sustainability of Wine Production*. Sustainability, 2020. **12**(2): p. 559.
44. Orth, U.R., L. Lockshin, and F. d'Hauteville, *The global wine business as a research field*. International Journal of Wine Business Research, 2007. **19**(1): p. 5-13.
45. Estreicher, S.K., *Wine: From Neolithic Times to the 21st Century*. 2006: Algora Pub.
46. Jackson, R.S., *1 - Introduction*, in *Wine Science (Third Edition)*, R.S. Jackson, Editor. 2008, Academic Press: San Diego. p. 1-14.

47. Fiore, M., et al., *Understanding the relationship between green approach and marketing innovations tools in the wine sector*. Journal of Cleaner Production, 2017. **142**: p. 4085-4091.
48. Robinson, J. and J. Harding, *The Oxford companion to wine*. 2015: American Chemical Society.
49. Sellers, R., *Would you Pay a Price Premium for a Sustainable Wine? The Voice of the Spanish Consumer*. Agriculture and Agricultural Science Procedia, 2016. **8**: p. 10-16.
50. Venkitasamy, C., et al., *Chapter 6 - Grapes*, in *Integrated Processing Technologies for Food and Agricultural By-Products*, Z. Pan, R. Zhang, and S. Zicari, Editors. 2019, Academic Press. p. 133-163.
51. Jackson, R.S., *9 - Specific and Distinctive Wine Styles*, in *Wine Science (Third Edition)*, R.S. Jackson, Editor. 2008, Academic Press: San Diego. p. 520-576.
52. Spigno, G., L. Marinoni, and G.D. Garrido, *1 - State of the Art in Grape Processing By-Products*, in *Handbook of Grape Processing By-Products*, C.M. Galanakis, Editor. 2017, Academic Press. p. 1-27.
53. Mateo, J.J. and S. Maicas, *Valorization of winery and oil mill wastes by microbial technologies*. Food Research International, 2015. **73**: p. 13-25.
54. Barba, F.J., et al., *Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: A review*. Trends in Food Science & Technology, 2016. **49**: p. 96-109.
55. Ruggieri, L., et al., *Recovery of organic wastes in the Spanish wine industry. Technical, economic and environmental analyses of the composting process*. Journal of Cleaner Production, 2009. **17**(9): p. 830-838.
56. Mandade, P. and E. Gnansounou, *Chapter NINETEEN - Potential value-added products from wineries residues*, in *Biomass, Biofuels, Biochemicals*, G.S. Murthy, et al., Editors. 2022, Elsevier. p. 371-396.
57. Hogervorst, J.C., U. Miljić, and V. Puškaš, *Extraction of Bioactive Compounds from Grape Processing By-Products*, in *Handbook of Grape Processing By-Products: Sustainable Solutions*. 2017. p. 105-135.
58. Dávila, I., et al., *2 - The Biorefinery Concept for the Industrial Valorization of Grape Processing By-Products*, in *Handbook of Grape Processing By-Products*, C.M. Galanakis, Editor. 2017, Academic Press. p. 29-53.
59. Velázquez-Martí, B., et al., *Quantification of the residual biomass obtained from pruning of vineyards in Mediterranean area*. Biomass and Bioenergy, 2011. **35**(8): p. 3453-3464.
60. Fiore, E., B. Stabellini, and P. Tamborrini, *A systemic design approach applied to rice and wine value chains. The case of the innovaecofood project in piedmont (italy)*. Sustainability (Switzerland), 2020. **12**(21): p. 1-28.
61. Beres, C., et al., *Towards integral utilization of grape pomace from winemaking process: A review*. Waste Management, 2017. **68**: p. 581-594.
62. *ISO 14000 Family - Environmental Management*.
63. 14001, I., *Environmental management systems Requirements with guidance for use 2015*. 2015.
64. Martins, A.A., et al., *Towards sustainable wine: Comparison of two Portuguese wines*. Journal of Cleaner Production, 2018. **183**: p. 662-676.
65. OIV, *Resolution CST 1/2008—OIV Guidelines for Sustainable Vitiviniculture: Production, Processing and Packaging of Products*. 2008.
66. FIVS, *Global Wine Producers Environmental Sustainability Principles*. 2016.
67. Baiano, A., *An overview on sustainability in the wine production chain*. Beverages, 2021. **7**(1).
68. Castellucci, F., *Resolution CST 1/2008. OIV Guidelines for sustainable vitiviniculture: production, processing and packaging of products*, 2008.
69. Puckette, M., *Better Than Organic: Sustainability and Wine*.

70. Felix, C.B., et al., *COVID-19 and industrial waste mitigation via thermochemical technologies towards a circular economy: A state-of-the-art review*. Journal of Hazardous Materials, 2022. **423**: p. 127215.
71. Ncube, A., et al., *Upgrading wineries to biorefineries within a Circular Economy perspective: An Italian case study*. Science of The Total Environment, 2021. **775**: p. 145809.
72. Ubando, A.T., C.B. Felix, and W.-H. Chen, *Biorefineries in circular bioeconomy: A comprehensive review*. Bioresource Technology, 2020. **299**: p. 122585.
73. *State of Food and Agriculture 2019. Moving forward on food loss and waste reduction*. 2019: FAO.
74. OIV *Managing by-products of vitivincultural origin* 2018.
75. Iuga, M. and S. Mironeasa, *Potential of grape byproducts as functional ingredients in baked goods and pasta*. Comprehensive Reviews in Food Science and Food Safety, 2020. **19**(5): p. 2473-2505.
76. Bevilacqua, N., et al., *Determinazione dell'impronta carbonica dei sottoprodotti della vinificazione e loro valenza biologica*. Ingegneria Dell'Ambiente, 2017. **4**(3): p. 277-285.
77. Bustamante, M.A., et al., *Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry*. Waste Management, 2008. **28**(2): p. 372-380.
78. Mora-Garrido, A.B., et al., *Revalorization of residues from the industrial exhaustion of grape by-products*. LWT, 2022. **156**.
79. Antonić, B., et al., *Grape Pomace Valorization: A Systematic Review and Meta-Analysis*. Foods, 2020. **9**(11): p. 1627.
80. Ferreira, S.M. and L. Santos, *A potential Valorization Strategy of Wine Industry By-Products and Their Application in Cosmetics—Case study: Grape Pomace and Grapeseed*. Molecules, 2022. **27**(3).
81. Beres, C., et al., *Antioxidant dietary fibre from grape pomace flour or extract: Does it make any difference on the nutritional and functional value?* Journal of Functional Foods, 2019. **56**: p. 276-285.
82. Zhu, F., et al., *Advance on the bioactivity and potential applications of dietary fibre from grape pomace*. Food Chemistry, 2015. **186**: p. 207-212.
83. Troilo, M., et al., *Grape Pomace as Innovative Flour for the Formulation of Functional Muffins: How Particle Size Affects the Nutritional, Textural and Sensory Properties*. Foods, 2022. **11**(12): p. 1799.
84. Beres, C., et al., *Antioxidant dietary fibre recovery from Brazilian Pinot noir grape pomace*. Food Chemistry, 2016. **201**: p. 145-152.
85. Baca-Bocanegra, B., et al., *Influence of Wine pH and Ethanol Content on the Fining Efficacy of Proteins from Winemaking By-Products*. Foods, 2022. **11**(12): p. 1688.
86. Davidov-Pardo, G. and D.J. McClements, *Nutraceutical delivery systems: Resveratrol encapsulation in grape seed oil nanoemulsions formed by spontaneous emulsification*. Food Chemistry, 2015. **167**: p. 205-212.
87. Troilo, M., et al., *Bioactive compounds from vine shoots, grape stalks, and wine lees: Their potential use in agro-food chains*. Foods, 2021. **10**(2).
88. Ruiz-Moreno, M.J., et al., *Valorization of grape stems*. Industrial Crops and Products, 2015. **63**: p. 152-157.
89. Kosińska-Cagnazzo, A., et al., *Phenolic compounds of grape stems and their capacity to precipitate proteins from model wine*. Journal of Food Science and Technology, 2020. **57**(2): p. 435-443.
90. Vázquez-Armenta, F.J., et al., *Antibacterial and antioxidant properties of grape stem extract applied as disinfectant in fresh leafy vegetables*. Journal of Food Science and Technology, 2017. **54**(10): p. 3192-3200.
91. Gouvinhas, I., et al., *Monitoring the antioxidant and antimicrobial power of grape (*Vitis vinifera* L.) stems phenolics over long-term storage*. Industrial Crops and Products, 2018. **126**: p. 83-91.

92. Engel, J.B., A. Ambrosi, and I.C. Tessaro, *Development of biodegradable starch-based foams incorporated with grape stalks for food packaging*. Carbohydrate Polymers, 2019. **225**: p. 115234.
93. Molina-Alcaide, E., A. Moumen, and A.I. Martín-García, *By-products from viticulture and the wine industry: potential as sources of nutrients for ruminants*. Journal of the Science of Food and Agriculture, 2008. **88**(4): p. 597-604.
94. Borges, M.S., et al., *Enriched cereal bars with wine fermentation biomass*. Journal of the Science of Food and Agriculture, 2021. **101**(2): p. 542-547.
95. Ayar, A., et al., *Probiotic properties of ice creams produced with dietary fibres from by-products of the food industry*. International Journal of Dairy Technology, 2018. **71**(1): p. 174-182.
96. Hwang, J.-Y., Y.-S. Shyu, and C.-K. Hsu, *Grape wine lees improves the rheological and adds antioxidant properties to ice cream*. LWT - Food Science and Technology, 2009. **42**(1): p. 312-318.
97. Kandylis, P., D. Dimitrellou, and T. Moschakis, *Recent applications of grapes and their derivatives in dairy products*. Trends in Food Science & Technology, 2021. **114**: p. 696-711.
98. Alarcón, M., et al., *Effect of Wine Lees as Alternative Antioxidants on Physicochemical and Sensorial Composition of Deer Burgers Stored during Chilled Storage*. Antioxidants, 2020. **9**(8): p. 687.
99. Llobera, A. and J. Cañellas, *Dietary fibre content and antioxidant activity of Manto Negro red grape (Vitis vinifera): pomace and stem*. Food Chemistry, 2007. **101**(2): p. 659-666.
100. Mildner-Szkudlarz, S., et al., *White grape pomace as a source of dietary fibre and polyphenols and its effect on physical and nutraceutical characteristics of wheat biscuits*. Journal of the Science of Food and Agriculture, 2013. **93**(2): p. 389-395.
101. Bordiga, M., F. Travaglia, and M. Locatelli, *Valorisation of grape pomace: an approach that is increasingly reaching its maturity – a review*. International Journal of Food Science & Technology, 2019. **54**(4): p. 933-942.
102. Bender, A.B.B., et al., *Effects of micronization on dietary fiber composition, physicochemical properties, phenolic compounds, and antioxidant capacity of grape pomace and its dietary fiber concentrate*. LWT, 2020. **117**: p. 108652.
103. Saura-Calixto, F., *Dietary fiber as a carrier of dietary antioxidants: An essential physiological function*. Journal of Agricultural and Food Chemistry, 2011. **59**(1): p. 43-49.
104. Jackson, R.S., 6 - *Chemical Constituents of Grapes and Wine*, in *Wine Science (Third Edition)*, R.S. Jackson, Editor. 2008, Academic Press: San Diego. p. 270-331.
105. Leal, C., et al., *Recovery of bioactive compounds from white grape (Vitis vinifera L.) stems as potential antimicrobial agents for human health*. Saudi Journal of Biological Sciences, 2020. **27**(4): p. 1009-1015.
106. Bouarab Chibane, L., et al., *Plant antimicrobial polyphenols as potential natural food preservatives*. Journal of the Science of Food and Agriculture, 2019. **99**(4): p. 1457-1474.
107. Leal, C., et al., *Potential application of grape (Vitis vinifera L.) stem extracts in the cosmetic and pharmaceutical industries: Valorization of a by-product*. Industrial Crops and Products, 2020. **154**: p. 112675.
108. AOAC, *Official Methods of Analysis of Association of Analytical Chemistry International*. 21<sup>st</sup> ed. 2019, Gaithersburg Maryland: AOAC International.
109. Büchi Labortechnik, A.G., *Non protein nitrogen determination in milk according to the Kjeldahl method. Application note 050/2010*. 2010.
110. Machado, S., et al., *A study on the protein fraction of coffee silverskin: Protein/non-protein nitrogen and free and total amino acid profiles*. Food Chemistry, 2020. **326**.
111. Krul, E.S., *Calculation of Nitrogen-to-Protein Conversion Factors: A Review with a Focus on Soy Protein*. Journal of the American Oil Chemists' Society, 2019. **96**(4): p. 339-364.

112. Costa, A.S.G., et al., *Optimization of antioxidants extraction from coffee silverskin, a roasting by-product, having in view a sustainable process*. Industrial Crops and Products, 2014. **53**: p. 350-357.
113. Singleton, V.L. and J.A. Rossi, *Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents*. American Journal of Enology and Viticulture, 1965. **16**(3): p. 144-158.
114. Alves, R.C., et al., *Antiradical activity, phenolics profile, and hydroxymethylfurfural in espresso coffee: influence of technological factors*. J Agric Food Chem, 2010. **58**(23): p. 12221-9.
115. Benzie, I.F.F. and J.J. Strain, *The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay*. Analytical Biochemistry, 1996. **239**(1): p. 70-76.
116. Mora-Garrido, A.B., et al., *Revalorization of residues from the industrial exhaustion of grape by-products*. LWT, 2022. **156**: p. 113057.
117. Jin, Q., et al., *Compositional Characterization of Different Industrial White and Red Grape Pomaces in Virginia and the Potential Valorization of the Major Components*. Foods, 2019. **8**(12): p. 667.
118. Rivas, M.Á., et al., *Chemical Composition and Functional Properties of Dietary Fibre Concentrates from Winemaking By-Products: Skins, Stems and Lees*. Foods, 2021. **10**(7): p. 1510.
119. Alonso, Á.M., et al., *Determination of Antioxidant Activity of Wine Byproducts and Its Correlation with Polyphenolic Content*. Journal of Agricultural and Food Chemistry, 2002. **50**(21): p. 5832-5836.
120. Blackford, M., et al., *A review on stems composition and their impact on wine quality*. Molecules, 2021. **26**(5).
121. Deng, Q., M.H. Penner, and Y. Zhao, *Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins*. Food Research International, 2011. **44**(9): p. 2712-2720.
122. Mohamed Ahmed, I.A., et al., *Chemical composition, bioactive compounds, mineral contents, and fatty acid composition of pomace powder of different grape varieties*. Journal of Food Processing and Preservation, 2020. **44**(7): p. e14539.
123. Mamma, D., et al., *Biotechnological potential of fruit processing industry residues, in Biotechnology for agro-industrial residues utilisation*. 2009, Springer. p. 273-291.
124. Stephen, A.M., et al., *Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health*. Nutrition Research Reviews, 2017. **30**(2): p. 149-190.
125. Alfaia, C.M., et al., *Use of Grape By-Products to Enhance Meat Quality and Nutritional Value in Monogastrics*. Foods, 2022. **11**(18): p. 2754.
126. Pascual, O., et al., *Influence of Grape Seeds and Stems on Wine Composition and Astringency*. Journal of Agricultural and Food Chemistry, 2016. **64**(34): p. 6555-6566.
127. Albuquerque, B.R., et al., *Phenolic compounds: current industrial applications, limitations and future challenges*. Food & Function, 2021. **12**(1): p. 14-29.
128. Alara, O.R., N.H. Abdurahman, and C.I. Ukaegbu, *Extraction of phenolic compounds: A review*. Current Research in Food Science, 2021. **4**: p. 200-214.
129. Zhishen, J., T. Mengcheng, and W. Jianming, *The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals*. Food Chemistry, 1999. **64**(4): p. 555-559.
130. Zhong, Y. and F. Shahidi, *12 - Methods for the assessment of antioxidant activity in foods*, in *Handbook of Antioxidants for Food Preservation*, F. Shahidi, Editor. 2015, Woodhead Publishing. p. 287-333.
131. Costa, A.S.G., et al., *Nutritional, chemical and antioxidant/pro-oxidant profiles of silverskin, a coffee roasting by-product*. Food Chemistry, 2018. **267**: p. 28-35.

132. Ky, I., et al., *Wine by-Products: Phenolic characterization and antioxidant activity evaluation of grapes and grape pomaces from six different French grape varieties*. *Molecules*, 2014. **19**(1): p. 482-506.
133. Tournour, H.H., et al., *Effect of Touriga nacional Grape Extract on Characteristics of Mechanically Deboned Chicken Meat Kept Under Frozen Storage*. *Journal of Food Process Engineering*, 2017. **40**(3).
134. Ghendov-Mosanu, A., et al., *Chemometric Optimization of Biologically Active Compounds Extraction from Grape Marc: Composition and Antimicrobial Activity*. *Molecules*, 2022. **27**(5).
135. Daglia, M., *Polyphenols as antimicrobial agents*. *Current Opinion in Biotechnology*, 2012. **23**(2): p. 174-181.
136. Radulović, N.S., et al., *Antimicrobial plant metabolites: structural diversity and mechanism of action*. *Curr Med Chem*, 2013. **20**(7): p. 932-52.
137. Gyawali, R. and S.A. Ibrahim, *Impact of plant derivatives on the growth of foodborne pathogens and the functionality of probiotics*. *Applied Microbiology and Biotechnology*, 2012. **95**(1): p. 29-45.
138. Hoss, I., et al., *Valorization of wine-making by-products' extracts in cosmetics*. *Cosmetics*, 2021. **8**(4).
139. Esparza, I., et al., *Phenolic composition of grape stems from different spanish varieties and vintages*. *Biomolecules*, 2021. **11**(8).