

Vinification By-products and Their Phenolic Compounds

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Abstract The waste production by grape industrialization has been growing because about 60% of the harvested fruits are used in vinification. Grape marc (skins and seeds) and lees (sediment solids) are the most abundant by-products of the winemaking process, because the former represents approximately 5% of the total grapes processed and the second, 4% of the total volume of wine produced. Some studies have shown that winery residues contain phenolic compounds, potent antioxidants that were not fully transferred to the wine during the winemaking process, thus have economic and functional interest.

Keywords: by-products, grape marc, lees, phenolic compounds, grape, winemaking

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1. Introduction

The generation of waste by the wine industry has grown because part of the planted grapes are destined for wine production, which generates about 5% waste in the form of grape marc, in relation to produced grapes, and 4% as lees, in relation to the produced wine. One of the wine residues generated in greater amounts is the grape marc. It consists mainly of skin and seed that are generated after concomitant maceration, alcoholic fermentation, and from grape pressing (in red wine or after pressing of the previously crushed grapes in the production of white wine). After fermentation of the must, the lees are obtained by decantation consisting of fine particles of grape residue and dead yeast. To date, viticulture industry data shows that these residues are being used as animal feed, as fertilizer, in as a source for alcohol distillation, as raw material for pharmaceutical and food industries. This explains the growing interest in exploring nobler forms of winemaking usage, as in the extraction of phenolic compounds [1,2,3].

Thus, this review aims to demonstrate the main phenolic compounds present in winery residues, which include phenolic acids, flavan-3-ols, flavonols and anthocyanins. Due to their importance to health and by the potential to be used as active compounds by pharmaceutical and food industry, this work provide relevant background information to future researches in the feasibility of winery waste as raw material.

2. Viticulture

Thousands of grape varieties exist in the world. Most of them belong to the species *Vitis vinifera*, of European origin, used for the production of fine wines. Moreover, others species classified as *Vitis labrusca* and *Vitis bourquina* are American grapes that are available *in natura* for fresh consumption or to be used as raw material for production of table wines. Thus, viticulture is considered as having great diversity with cultivars of *Vitis vinifera*, American grapes (*Vitis labrusca* and *Vitis bourquina*), and interspecific hybrid grapes. Some of them are consecrated for the wide adaptability and for the characteristics of the wines produced; others, have a more restricted adaptation, remaining in their regions of origin, catering for the preparation of typical and unique products [4].

3. Winery Waste

The grape wine industries produce large amounts of agro-industrial waste. With this problem, the search for alternatives in the use of the generated organic matter is growing within several research centers [5].



Figure 1. Grape marc BRS Lorena (A) and grape marc Cabernet franc (B)

The grape marc (Figure 1) is the most abundant of residues, produced after the pressing of the grapes in the production of white wine, or after the maceration/alcoholic fermentation step during the production of red wine. The lees consist of fine particles of grape residue and inactive yeasts that are obtained by decantation after the process of must fermentation [2,3].

Lees (Figure 2) are regarded as the residues that accumulate in wine vessels after fermentation. This phenomenon occurs during storage or after authorized treatment and is separated by filtering and/or centrifuging this product. This residue is mainly composed of micro-organisms (yeast), poorly soluble salts of tartaric acid (such as potassium and calcium bitartrate), inorganic matter and phenolic compounds [6].

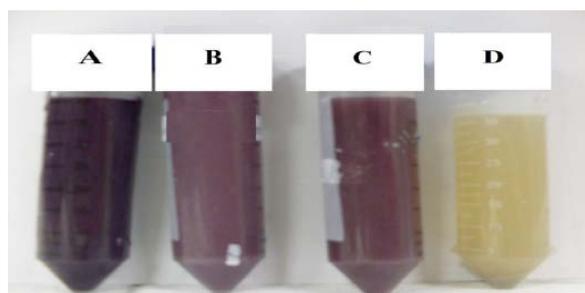


Figure 2. Lees BRS Violeta (A), Cabernet sauvignon (B), Cabernet franc (C) and BRS Lorena (D)

The quantities of lees obtained depend on several factors; those inherent of the grape constitution, ripeness, and hygiene state of the berries. Climatic conditions and the winemaking techniques adopted aren't able to establish a precise relation with the amount of lees generated [7].

According to industry data, the waste generated in wine production is usually used as animal feed, even with the inconvenience of the presence of alcohol, and as vineyard fertilizer, with the grape marc also being used in distilling in the alcohol winery. However, most of this waste is still disposed without any previous treatment resulting in damage to the environment as, for example, the contamination of surface and ground water [8,9].

However, the use of these wine industry residues appropriately could provide a balance in the environment. The large amounts of waste produced by agro-industries cause serious damage, as already mentioned [5,8,10].

The disposal of these residues, as is done today, causes an economic deficit in the supply chain, since many of these residues are rich in bioactive compounds, i.e. compounds with antioxidant powers among other benefits like antimicrobial activity and cardiovascular health benefits. Therefore they have a high commercial value, awakening scientific and economic interest. Among the bioactive compounds, the waste generated by the wineries is a rich source of phenolic compounds since a significant amount results from the processing. The grape marc (skins and seeds), rachis and lees all together represent an average of about 30% of the volume of grapes used for wine production, which makes them a promising source of natural bioactive substances with high added value [11]. This explains the increasing interest in exploring the phenolic compounds such as antioxidants present in wine waste by virtue of their functionality.

Previous studies with the grape marc have demonstrated that it contains compounds which were not completely removed during the winemaking process, because it has a high amount of secondary metabolites of grape, including phenolic acids, flavan-3-ols and anthocyanins [12].

A vast majority of studies in the literature are conceptualized in the study of residual phenolic compounds. A part of these compounds is transferred to wine, however a greater part remains in waste, because these compounds are present in the solid parts of the grape and its extraction depends mainly on the technological conditions used during winemaking such as: maceration time, temperature, intensity and pressure duration, use of enzymes, type of yeast and SO₂ concentration [12,13].

Amico et al. [14], Arvanitoyannins et al. [10] and Amendola, Favari and Spigno [15] presented a study reporting the functional properties of the wine residue, specifically the analyzed grape marc, and the main compounds found were phenolic compounds, anthocyanins, catechins, tartaric acid, malic acid, sugars, fatty acids and minerals. Kammerer, Claus Carle and Schieber [16] characterized the phenolic compounds of grape marc produced in Germania, and identified 13 anthocyanins, 11 phenolic acids, catechins and stilbene. Karvela, Makris, Kalogeropoulos, Karathanos and Kefalas [17] found gallic acid, tannic acid, catechin and dimers and flavanols in wine seed residues in Greece.

Studies on wine grape marc demonstrate large variations in the content of phenolic compounds found, ranging from 0.1 to 7.0 g of gallic acid equivalent/100 g of dry grape marc. This oscillation is mainly due to the different varieties of grapes used, the diverse winemaking techniques, and different methods of analysis for this type of waste [5,9,15,18].

4. Phenolic Compounds

The vine (*Vitis* spp.), among many other vegetables, stands out due to the high levels of phenolic compounds present in the fruit tissues, leaves and seeds, as well as the variability of the chemical structures found. A growing number of studies have studied the qualitative/quantitative aspects of these compounds in biomass of various species and varieties of vines and their products, such as wines and juices and other products [19,20,21].

Chemically, the phenolic compounds belong to a class of molecules with large structural diversity, characterized by presenting an aromatic ring, having one or more hydroxyl groups directly linked with the ring structure, and may range from simple molecules to highly polymerized phenolic compounds [22].

Phenolic compounds are secondary metabolites that do not participate in metabolic pathways responsible for growth and reproduction, so, their nature and concentration vary greatly.

They can be classified into two groups: flavonoids and non-flavonoids.

4.1. Flavonoids

Flavonoids are those which have the chemical structure described as C₆-C₃-C₆ consisting of two phenolic rings (A and B), linked by a central pyran (containing oxygen) ring

(C). Variations in replacing the C ring result in important classes of flavonoids such as flavonols, flavones, flavanones, flavanols (or catechins), isoflavones and anthocyanidins. The replacements of the A and B rings originate from different flavonoid compounds within each class [23].

Flavonoids are widely synthesized in the endoplasmic reticulum cell before being translocated and stored in the central vacuole of the producer cell. Their alleged role in grapes (and other plants) is like a line of defense against microbial pathogens, pests and herbivores and against changes in water content, light, UV radiation and mineral deficiency [24,25].

Flavonoids are formed from the combination of synthesized derivatives of phenylalanine (shikimic acid metabolic pathway) and acetic acid (acetyl-CoA). The group of flavonoids is also known as polyphenolic and usually occurs in plants in the form of glycosides, one of the classes of substances responsible for allocating the sensory profile. The distribution of flavonoids in the plant depends on many factors and can vary according to the order and the plant family, as well as the variation of species. As an example, one can mention the red grapes variety that are characterized by having higher contents of anthocyanins in the skin tissue, than the white and pink varieties [26].

Among the flavonoids, occurrence is mainly seen in the skins of red grapes, and to a lesser extent in the pulp of the dye varieties, being responsible for blue, violet, purple and red colors. Besides the possibility of being used as substitutes for synthetic dyes in food, the relationship between antioxidant capacity and possible beneficial health effects is also studied [27].

Anthocyanins are included in naturally occurring pigments group (Figure 3). Structurally, anthocyanins are glycosylated derivatives of 2-phenyl benzopyrilium cation, also called flavilic cation. The substitution at position 3 of the C ring and in position 5 and 7 of the A ring, by one or more sugar units in anthocyanidin (aglycone) results in anthocyanin. Structural variations of anthocyanins denote different linked sugars, of polymerization and of modes or positions of hydroxylation and methylation. In some cases, the sugars are shown to be acylated by *p*-coumaric and caffeic acids [28].

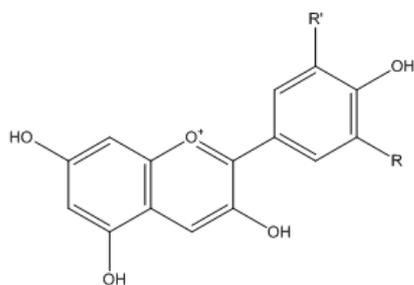


Figure 3. Basic structure of anthocyanidins [29]

In literature, 23 anthocyanidins have been mentioned in grapes, these differ from each other by the number and position of hydroxyl/methoxyl groups, although there is a predominance of 5 anthocyanins: cyanidin, delphinidin, peonidin, petunidin and malvidin, present both in wine and in non-wine grapes. Small amounts of perlagonidin were also found in wine grapes. Their relative amounts vary with the variety, however malvidin is usually the

most abundant in quantity. It is characteristic of *Vitis vinifera* to find only one glucose molecule attached at position 3 (3-glycoside), since other species of the genus *Vitis* also contain diglycosides at positions 3 and 5 (3,5-diglycosides), as seen in *Vitis labrusca* (for example, Concord), *Vitis rupestris*, *Vitis riparia*, and the Asian species *Vitis amurensis* which contain both 3-glycosides and 3,5-diglycosides, while the *Vitis rotundifolia* (Muscadine) grapes contain only 3,5-diglycosides [27,30]. The *V. vinifera* hybrids with non-wine grapes also contain a mixture of the anthocyanidins 3-glycoside and 3,5-diglycosides,

Unlike grapes, phenolic composition of wine, shows that about 25% anthocyanins may have been polymerized with flavonoids and other phenolic compounds during fermentation. This level can rise to over 40% within one year and may reach the level of 100% after several years [25].

In red wine made from *Vitis vinifera* grape cultivars, the pyranoanthocyanins are formed in the early stages of fermentation, mainly derived from the reaction of pyruvic acid with anthocyanins (anthocyanidin-3-glycosides) and acetaldehyde, two intermediary yeast metabolites [31]. So it is not surprising to find a kind of pyranoanthocyanin in the winemaking by-products, as reported for Sicilian cultivar grape marc Nerello Mascalese [14].

Barcia et al. [32] found 19 anthocyanins in the skin (from grape marc) of Cabernet sauvignon and Cabernet franc cultivars, along with 6 and 5 pyranoanthocyanins respectively, while in the lees 9 pyranoanthocyanins were identified along with 16 and 14 anthocyanins respectively for Cabernet sauvignon and Cabernet franc. These had free aglycones and acetylated and/or glycosylated aglycones. Barcia et al. [33] also found 27 anthocyanins in the skin (from grape marc) and 26 anthocyanins and 3 pyranoanthocyanins in the lees of BRS Violeta.

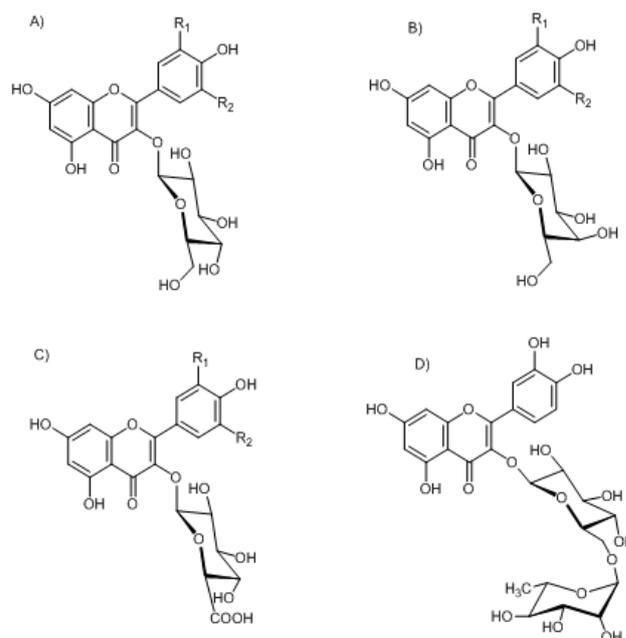


Figure 4. Structure of flavonols: kaempferol (R1=R2=H); quercetin (R1=OH, R2=H); isorhamnetin (R1=OCH3, R2=H); myricetin (R1=R2=OH); laricitrin (R1=OCH3, R2=OH); siringetin (R1=R2=OCH3). a) flavonol-3-O-glucoside; b) flavonol-3-O-galactoside; c) flavonol-3-O-glucuronide; d) rutin (quercetin-3-O-(6''-ramnosil)-glucoside) [35]

Castillo-Munoz, Gómez-Alonso, García-Romero and Hermosín-Gutiérrez [34] reported that the occurrence of flavonols in red grape cultivars is represented by six structures: kaempferol, quercetin, isorhamnetin, myricetin, laricitrin and siringetin (Figure 4).

A few years ago it was stated that only white grapes were methoxylated derivatives of flavonols disubstituted from ring B, such as kaempferol and quercetin. However, it has been demonstrated that the white grapes are all possible derivatives of flavonols with one or two substituents on ring B (kaempferol, quercetin, and isorhamnetin) with all tri-substituents (myricetin, laricitrin and siringetin) being absent in ring B [36,37]. Moreover, the predominant flavonols in *Vitis vinifera* are quercetin and myricetin on red grapes and quercetin only for white grapes, while in *Vitis labrusca* grapes it is quercetin [35].

The conjugates in grape flavonols are mainly 3-O-glycosides and the presence of sugars in other flavonol skeletal positions has never been reported. In *V. vinifera* grapes, other two complete minor series of glycosides are found as well, derivatives 3-galactoside and 3-glucuronides. 3-glucuronide of quercetin is an exceptional case since it is usually one of the most important flavonols, both in red and white grapes. Furthermore, in the case of quercetin, small proportions of the derivative 3-rhamnosylglucoside (3-rutinoside, a disaccharide) are found, which for several years was confused with the 3-glucuronide quercetin, even affirming that it was the most abundant of flavonols [27]. In the case of non-wine varieties of red grapes, like isorhamnetin in grapes, only glucose derivatives have been identified, though myricetin and quercetin may also occur as glucuronide and galactoside, and myricetin derivatives are often in these cases the majority [38].

In Brazilian winery wastes, about 18 flavonols were found in the skins of Cabernet sauvignon and Cabernet franc cultivars and on average 15-16 flavonols, in the lees, respectively [32]. In the wastes of hybrid cultivars (BRS Violet and BRS Lorena) an average of 12 flavonols were identified [33].

The flavan-3-ols are compounds found in higher quantities in grape seeds, and also importantly in skins. The combination of several monomeric units of catechins and epicatechins are called proanthocyanidins or condensed tannins, these are not easily hydrolysable. In the case of varieties of *Vitis vinifera*, the main elements are monomeric forms of (+) - catechin and (-) - epicatechin and mostly, their oligomers and polymers [30]. Proanthocyanidins in skins and seeds show different structural features. The proanthocyanidins from the skin have a higher degree of polymerization and contain prodelfinidins (structural units with 3 substituents on ring B, such as gallo catechin and epigallo catechin), while the seeds have a lower degree of polymerization and do not contain prodelfinidins and have relevant amounts of gallic esters (such as 3-gallate of epicatechin) [39]. Among the phenolic compounds, high molecular weight tannins have the ability to combine with proteins or other polymers such as polysaccharides, causing the sensation of astringency. The low molecular weight tannins on the other hand tend to provide a bitter taste [40].

4.2. Non-Flavonoids

The non-flavonoids consist of phenolic acids, hydrolysable tannins, and stilbenes ($C_6-C_2-C_6$) [5,23].

The small amounts of phenolic material found mainly in white wines are not flavonoids (hydroxycinnamic), such as caftaric, *p*-coumaric and ferulic acid [25], due to limited maceration with the solid parts of the grape (skins in this case) occurring when obtaining the must, that leads to the end of the fermentation.

The hydroxybenzoic acids include gallic acid, *p*-hydroxybenzoic, protocatechuic, vanillic and syringic, which have the common C_1-C_6 structure; while the hydroxycinnamic acids have a side chain with three carbon atoms (C_6-C_3), they are vinyl-analogs of hydroxybenzoic acid, such as caffeic, ferulic, sinapic and *p*-coumaric acids [41].

Hydroxycinnamic acids (HCA) are representatives of the classes of phenolic acids found in grapes and wine. The main HCA found in grapes and wine are combined with tartaric acid (hydroxycinnamoyl-tartaric acids), which are called caftaric acid, *p*-cutaric acid and fertaric acid, derived from caffeic, *p*-coumaric and ferulic acids, respectively (Figure 5).

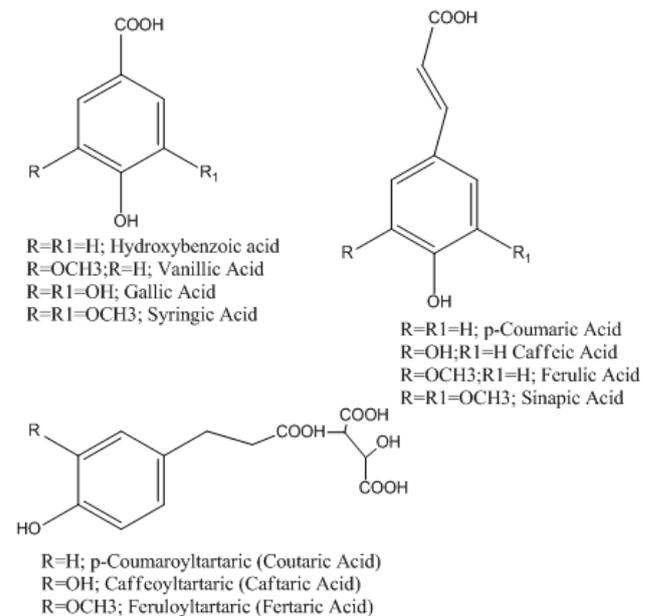


Figure 5. Structure of phenolics acids [42]

Typically caftaric, cutaric and fertaric acids are found in the pulp of grapes in the *trans* form, and are released during grape pressing, while *p*-cutaric acid is presented in an appreciable manner in *cis*-form, with the *cis* isomers of caftaric and tartaric acids being practically non-existent. Thus, they remain in the wine residues and are associated with the wine darkening process and are precursors of volatile phenolic compounds. During the wine fermentation process, the partial hydrolysis of these types of esters yields the free hydroxycinnamic acids. These are converted into ethyl ester, or ethyl caffeate and ethyl coumarate [42], and can also be decarboxylated by yeasts and bacteria (4-forming vinylphenols), and even later transformed into volatile ethylphenols of unpleasant aroma by *Brettanomyces* type yeast.

In Cabernet sauvignon and Cabernet franc cultivars, 4 to 5 derivatives of hydroxycinnamic acids (HCA) were identified from the skin, and 5 to 6 HCA in the lees, respectively [32]. And in hybrid cultivar wastes (BRS

Violet and BRS Lorena) 7 to 8 HCA derivatives were found [33].

Besides these compounds present in the grape, resveratrol, a polyphenol belonging to the class of stilbenes is also found. Resveratrol (3,5,4'-trihydroxystilbene) is a phenolic compound that comprises two aromatic rings linked by an ethylene bridge. It is synthesized in the plant using an enzyme, stilbene synthase, which combines a molecule of hidroxycinnamoil-Coenzyme A (CoA) and three molecules of malonyl-CoA in two isomeric forms: *trans*-resveratrol and *cis*-resveratrol (Figure 6).

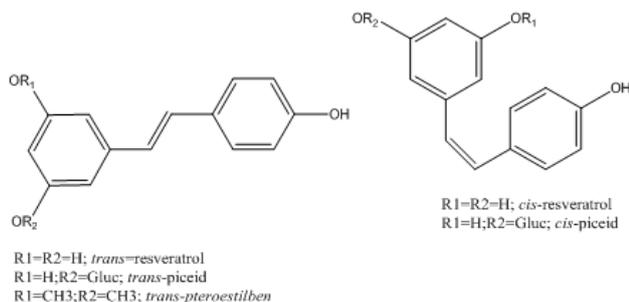


Figure 6. Structure of resveratrol [42]

The *trans* form is the true form produced in a biosynthetic pathway and is glycosylated at position 3 (3-glucoside *trans*-resveratrol, also known as *trans*-piceid) [43,44]. The *cis* isomer may already be formed in grapes by induction of UV radiation. The piceid is also hydrolyzed in the grape and later in wine releasing resveratrol. Resveratrol has been identified in wine, the vine leaves and the skin of grapes, and its concentration decreases significantly during grape ripening [42].

Modifications in resveratrol, such as the already described glycosylation, methylation and polymerization, produce piceid, and Pterostilbene and viniferins. The *trans*-resveratrol has attracted special attention because of their spatial conformation that can activate calcium absorption by the blood vessels, triggering vasodilation, which contributes to lowering blood pressure, a fact demonstrated by epidemiological studies, which claim inverse correlation between moderate wine consumption and the incidence of cardiovascular disease [40].

In Brazilian wineries, waste such as the lees of Cabernet sauvignon and Cabernet franc, only 3 stilbenes were identified, among them: *trans*-piceid, *cis*-piceid and *trans*-resveratrol [32]. The same stilbenes were identified in wine residue shells of BRS Lorena [33].

The hydrolysable tannin result from binding of a sugar, usually glucose, to a phenolic compound, particularly gallic acid or ellagic acid, thereby forming gallotannins and ellagitannins, respectively. These compounds do not contain flavonoid molecules and are not found naturally in grapes. However, they are present in the wood used in storage or aging wines and therefore can be transferred to the wine and waste during these processes.

5. Trends and Prospects

With the data presented in this study, it was observed that there is concern about the destination of the waste generated by the wine industry. This waste has been

studied by virtue of the presence of bioactive compounds of great value.

Previous studies have shown that the grape marc, consisting of skins and seeds, and lees present considerable amounts of phenolic compounds with potential health promoting properties.

Because of this, the recovery of phenolic compounds from industrial waste is gaining attention, especially attributed to the functional properties that these compounds exert, such as anti-inflammatory, antimutagenic and anticancer.

With the work currently being performed, and with the results obtained that show that the wine by-products have the potential to be applied in food, pharmaceutical and chemical industries, the recovery of by-products generated is expected to grow in coming years and that may solve the problem of the generation of industrial waste and provide consumer health benefits.

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