INFLUENCE OF ENVIRONMENTAL AND TECHNOLOGICAL PARAMETERS ON PHENOLIC COMPOSITION IN RED WINE

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Abstract

This review discusses some of the parameters that influence the content of polyphenols in red wine. Best quality grapes, sunny weather and late harvest increases the total phenolic content in wine as well as high-phenolic content grape cultivar. Nevertheless, if the extraction technique is not efficient the resulting wine will be poor in these compounds. Mash heating followed by fermentation on skin, pre-fermentating enzymatic treatment and long maceration time are processes increasing the concentration of polyphenols in the resulting wine while oxygen supply during storage reduces the total content of low molecular weight phenolic compounds and increases the polyphenols polymers that only seems to stabilize the wine's color.

Riassunto

In questa review vengono presi in considerazione alcuni parametri che influenzano il contenuto dei polifenoli nel vino rosso. La qualità delle uve, il clima caldo, il raccolto tardivo, la cultivar ricca in polifenoli aumentano il contenuto di polifenoli totale nel vino. Tuttavia, se le tecniche estrattive non sono efficienti il vino che ne risulterà sarà povero di questi composti. La pressatura a caldo seguita dalla fermentazione con le bucce, il trattamento enzimatico pre-fermentativo ed il lungo tempo di macerazione sono processi che incrementano la concentrazione di polifenoli totali nel vino.

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Al contrario, la somministrazione di ossigeno durante la conservazione riduce il contenuto dei polifenoli a basso peso molecolare e aumenta i polimeri dei polifenoli che sembrano stabilizzare il colore del vino.

Key words: Red wine, Phenolic compounds, Environmental parameters, Wine making techniques, Quality

Introduction

For centuries wine has been considered as a fine beverage; Louis Pasteur said "wine is the most healthful and most hygienic of beverages". In 1987 Richard described a phenomenon popularly called the French paradoxe: French people compared to people from other industrial countries, are subjected to low incidence of coronary heart disease, despite having a diet rich in fat, blood cholesterol levels generally higher and being heavy smokers (1). After many epidemiological studies, wine consumption was negatively correlated to coronary artery disease mortality indicating a protective effect (2-6). Because wine alcohol consumption is more strongly correlated ($r = -0.66$) with reduced coronary disease mortality than total alcohol consumption referred as all other alcoholic beverages ($r = -0.39$), modern scientific research has focused on the non-alcoholic fraction of wine (7-9).

Wine possesses more than 500 substances but a major class of compounds differentiates from other alcoholic drinks: phenolic compounds (10). In particular, red wine have been more studied because its concentration in phenolics is much higher than that of white wine. These compounds have a numerous roles including UV protection, pigmentation, disease resistance, and nodule protection (11,12).

Oenologists have always paid a particular attention to the phenols content because they substantially contribute to the quality of wine threw their sensory properties. These substances are responsible for wine's bitterness and astringency; they affect it's color, flavor, stability and aging behavior (13-19).

More recently, Sato (20) found a positive correlation between total phenolic content and radical scavenger activity of wine. Polyphenols contain a number of phenolic hydroxyl groups attached to a ring structure, conferring the antioxidant activity. This class of compounds can act as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers (21). The radical scavenging activity of polyphenols in vitro has proven to have a more effective antioxidant power than vitamins E and C on a molar basis (22, 23). Phenolic compounds have also been shown to be powerful inhibitors of low density lipoproteins oxidation (24,25). Moreover, recent clinical studies have demonstrated that the consumption of red wine increases the antioxidant capacity of human plasma (26-30).

Phenolic compounds are ubiquitous in plant kingdom and are secondary metabolites, meaning they don't have a direct impact on the physiological plant functions like growth and reproduction (31). The specific expression of each cultivar is the dominating factor affecting the phenolic composition of the resulting wine. On the other hand, concentration and distribution of the different compounds in red wine grape can be influenced by environmental, technical and management parameters such as season, grape harvest, weather, agricultural practices, diseases, and extraction technologies.

Flavonols, particularly when they occur in their deglycosylated form, are labile molecules and may be degraded upon exposure to heat (32), enzymes (33), and oxidative chemical species, such as free radicals (34). Therefore, it would appear reasonable that processing and other treatments of grapes and grape products might afford prominent alteration in the flavonol profile. In the case of wines, common vinification practices, including skin contact, stabilization processes and ageing, are responsible for significant changes in flavonols, from both a qualitative and a quantitative point of view.

Factors that may profoundly differentiate flavonol composition are also those associated with ageing and storage conditions. Oxygen seems to play a central role, as supplementation with oxygen during storage decreased quercetin levels by more than 50% over a period of 6 months (35). Another study concerned with the evolution of flavonols upon storage in barrels made from different types of wood indicated that losses of both glycosides and aglycones were significantly more pronounced in barrels made of American oak, in comparison with barrels made of French and Spanish oaks (36).

This finding highlighted the impact of the wooden container on the relevant oxidative reactions, since the levels of oxygen that may come into contact with the wine through the staves largely depend on the size of wood pores. Temperature is another determinant of flavonol evolution, and it was shown that quercetin levels were always lower in samples stored at 22°C than those at 12 °C (37). All these aspects account for the great variability of red wine phenolic profile, evaluated on the basis of literature data.

Chemical structure of wine's polyphenols

Phenolic compounds are classified in two main groups (Figure 1-2): non flavonoids and flavonoids. The non flavonoids, phenols with only one aromatic ring (C6), include the benzoic acids derivatives (C6-C1), and the cinnamic acid derivatives (C6-C3). Another class of non-flavonoids the stilbenes has attracted much interest lately because of their biological properties (38-46). Their basic squeleton is formed by two aromatic rings joined by a methylene bridge (C6-C3-C6). In contrast, flavonoids are a large class of compounds that have the same skeleton, the flavane nucleus, consisting of two benzene rings linked by an oxygen containing pyrane ring (C6-C3-C6). The classification is done according to the oxygenation state of the heterocycle. This class of compounds is divided in two main groups: the anthocyanins and the anthoxanthins. The anthoxanthins comprise the flavones, flavanones and flavonols.

As we seen in table 1, the individual difference within each group result from the variation in number and arrangiamnt of the hydroxyl group as well as from the nature and extend of alchilation and glicosilation of these group. The most commonly occurring flavones and flavonols are those with dihydroxilation in the 3' and 4' position of the ring B, and a lesser extent, those with a lone B ring-hydroxyl group in the 4' position. While the preferred glycosilation site on the flavonoids is the 3 position and less frequesntly the 7 position.

The chemical properties of poliphenols in terms of availabilty of the phenolic hydrogens as hydrogen-donating radical scavengers predict their antioxidant activity. For a poliphenols to be defined as an antioxidant it must satisfy two basic conditions: first, when present in low concentration relative to the substrate to be oxidized it can delay, retard, or prevent the autoxidation or free radical-mediated oxidation; second, the resulting radical formed after scavenging must be stable-through intra-molecular hydrogen bonding on further oxidation (23).

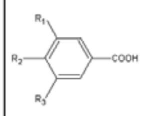
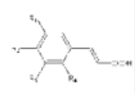
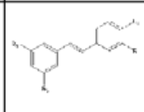
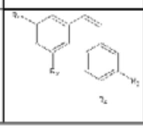
Non Flavonoids	Chemical structure	Name	R1	R2	R3	R4
Benzoic acid		Gallic acid	OH	OH	OH	
		Protocatechinic acid	H	OH	OH	
		Vanillic acid	H	OH	OCH_3	
		Syringic acid	OH	OCH_3	OCH_3	
Cinnamic acid		Ferulic acid	H	H	OH	OCH_3
		p-cumaric acid	H	H	OH	H
		Cinnamic acid	H	H	H	H
		Caffeic acid	H	H	OH	H
Trans-stilbenes		*Trans*-astringin	Glucose	OH	OH	OH
		Trans-piceid	Glucose	OH	H	OH
		Trans-resveratrol	OH	OH	H	OH
Cis-stilbenes		*Cis*-piceid	Glucose	OH	H	OH
		Cis-resveratrol	OH	OH	H	OH

Fig. 1 - Chemical structure of the most common non-flavonoids polyphenols in wine.

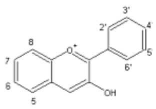
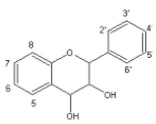
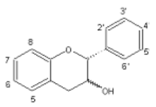
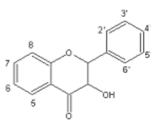
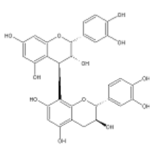
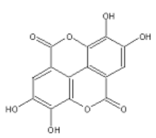
Flavonoids	Chemical structure	Name	5	7	3’	4’	5’
Anthocyanidins		**Pelargonidin**	**OH**	**OH**	**H**	**OH**	**H**
		Cyanidin	**OH**	**OH**	**OH**	**OH**	**H**
		Peonidin	**OH**	**OH**	**OCH_3**	**OH**	**H**
		Delphinidin	**OH**	**OH**	**OH**	**OH**	**OH**
		Petunidin	**OH**	**OH**	**OCH_3**	**OH**	**OH**
		Malvidin	**OH**	**OH**	**OCH_3**	**OH**	**OCH_3**
Flavan 3,4-diols		**Leucopelargonodin**	**H**	**H**	**H**	**H**	**H**
		Leucodelphinidin	**H**	**H**	**OH**	**H**	**OH**
		Leucocyanidin	**H**	**H**	**OH**	**H**	**H**
Flavan-3-ols		**Catechin**	**OH**	**OH**	**OH**	**H**	**H**
		Epicatechin	**OH**	**OH**	**OH**	**H**	**H**
Flavonols		**Kaempferol**	**OH**	**OH**	**H**	**OH**	**H**
		Quercetin	**OH**	**OH**	**OH**	**OH**	**H**
		Myricetin	**OH**	**OH**	**OH**	**OH**	**OH**
Condensed tannins		**C-C bond or C-O bond of catechin, epicatechin and leucocyanidin**					
Hydrolyzable tannins		**The major source of these tannins come from oak extraction (ellagic acid)**					

Fig. 2 - Chemical structure of the most common flavonoids polyphenols in wine

Effect of grape cultivar, quality and geographical origins on the phenolic content of red wines

Few litterature has been able to show the influence of the vitis cultivar differences on the content of polyphenols in red wines, because it would require grape growth under the same geographical region at the same time.

Nevertheless Soleas and coworkers, examined the composition of wines made from different cultivars grown in the same regulated viticulture region of Niagara, Southern Ontario, Canada, and processed by the same production techniques.

This was to determine if significant and characteristic difference in content and relative patterns of individul polyphenols could be identified among the wines of varous coultivar. An assay involving solid phase extraction followed by derivatization and gas chromatogaphy/mass spttrometry was used to quantify the polyphenols.

They measured in red wine 10 classes of poliphenols: gentisic, vanillic, ferulic, p-cumaric, caffeic, gallic acid, cis and trans resveratrol, cis and trans polydatin, catechin and epicatechin and quercetin. This work did not suffer distortion from environmental and enological factors.

Five red wine grapes cultivar were analysed: Gamay Noir, Merlot, Cabernet Sauvignon, Cabernet Franc and Pinot Noir.

Within total phenolic acids, cultivar related differences were noticed for caffeic acid content that ranged from 3.15 mg/l for Merlot to 12.95 mg/l for Cabernet Sauvignon. The other phenolic acids suffered equal variations: gentisic acid mean concentrations 0.44mg/l-0.46 mg/l; vanillic acid ranging from 2.3 mg/l for Gamay Noir to 3.7 mg/l Pinot noir; ferulic acid in all red wines concentrated < 1.0 mg/l except Cabernet Franc, which had a mean concentration of 2.86 mg/l; p-cumaric acid mean concentrations 2.61 mg/l for Pinot Noir to 4.5 mg/l for Gamay Noir; gallic acid ranging from 13.08 mg/l for Gamay Noir to 30.67 mg/l for Pinot Noir; cis- and trans resveratrol isomers was found higher in Merlot wines 3.20 mg/l and lowest in Cabernet Franc 0.98 mg/l; cis and trans polydatin isomers ranged between 0.04 mg/l for Cabernet Franc and 1.68 mg/l Pinot Noir; catechin and epicatechin Pinot Noir had by far the highest mean concentrations of these compounds at 213 and 82 mg/l respectively; quercetin in Cabernet Sauvignon had a significantly higher mean concentration at 5.26 mg/l than all other red wines analyzed (47).

It was noticed that all red wines had very similar total phenolic acid concentrations, ranging from 0.200 mmol/l for Gamay Noir to 0.259 mmol/l for Pinot Noir.

Faustino and coworkers examined the level of catechin, epicatechin, rutin, quercetin and trans-resveratrol in Merlot wine; this wine is a variety that recently gained popularity in North America. They studied if weather and different cultivar origin (Chilean, Canadian and American) could influence the phenolic content, concluding that environment, temperature, humidity and soil nutrients are only few examples of variable influencing phenolic composition of wines. Indeed, they found that catechins account for the majority of the phenolics in Chilean and Canadian wines (70 and 73% respectively), but epicatechins cover the majority of the phenolic analyzed in America wine (51%, 19% and 22% in Chilean and Canadian respectively). Quercetin was the third most abundant antioxidant in all three groups with rutin and trans-resveratrol accounting for less than 5% of phenolic content in all wine examined. American wines, however, possessed a significantly lower amount of trans-resveratrol than Merlots from the other two countries (48).

More recently, Prado and coworkers studied the influence of "terroir effect" on the feature of a red wine; this effect include weather, landscape (slope, exposure, biological and physiological environment), soil (depth, chemical composition, fertility and water availability) and geology. Moreover, they conducted a sensory evaluation and phenolic compound analysis as well as composition and stilbene concentration tests, conducted on two vineyard during two consecutive harvests in 2004 and 2005, in order to analyze the effect of soil on wine. They found that at the same grape ripening degree, soil might affect wine characteristics. Wines issued from the richer soil and with the less coarse fraction presented less total phenolic content and color intensity, but higher stilbene concentration. The influence of soil is stronger in a season with moderate rainfall (2004), compared to a season with low rainfall (2005) (49).

In table 1 is reported the concentration of phenolic compound in different red wine analyzed by several researches. Magarino and coworkers have studied the influence of the grape harvesting date (degree of maturity of the grape) on their chromatic characteristics and polyphenols content in two kinds of wine (Tinto Fino and Cabernet Sauvignon). The results showed that the harvesting data influence both the chromatic characteristics than the phenolic composition (50).

For quercetin, cultivar differences were noticed for Cabernet Sauvignon who expressed the highest content (5.25 mg/l). This grape cultivar is well known for it's high skin: volume ratio, thus high content of flavonol in the resulting red wines (table 2). In general, thick skin grapes make higher flavonol content of wine than other thin skin grapes like Grenache cultivar (51).

Harvest time is also an influential factor. For example Pinot Noir grapes are harvested later than the other cultivars and have a content of 2.60 mg/l and 0.5 mg/l respectively of quercetin. In countries where the weather is more stable, like Chile, than other cool, damp climate, like north of France and Italy, grapes are allowed to ripen longer and thus have a higher flavonol contents (52).

It is well known that the biosynthetic pathways involved in flavonoid production in plant tissue are greatly influenced by sunlight. Indeed, a detailed examination of sunlight exposure and temperature on the contents of quercetin, myceritin and kaempferol revealed that berries from sun exposed cluster might contain as much ten times the content found in sample obtained from shaded cluster (44). Sun exposition also plays an important role in the content of polyphenols in the red wine. Pinot Noir, which is a thin skin grape depending on the geographical region of growth the resulting wines have great flavonol content differences (table 3). This corroborates the conclusions of Karumanchiri and Price that higher sunlight exposition influences positively the content of quercetin in grape berries (53-54).

On the other hand grape quality has also high influence on the phenolic content of the resulting wine (55). With increasing grape quality the ratio of skin to volume is superior to that of lower quality grapes of the same cultivar because their berries size becomes smaller. Thus, for same volume of wine, a much greater volume of high quality grapes are required than that of lower quality fleshier grapes.

TABLE 1

POLYPHENOLIC CONTENT IN DIFFERENT WINES OBTAINED FROM LITERATURE

Phenolic compound	Content mg/l	Kinds of wine	References
Vanillic Acid	2.3- 3.7	Gamay Noir, Merlot, Cabernet Sauvignon, Cabernet Franc and Pinot Noir.	(47)
Ferulic Acid	<1.0 -2.86		
p-Cumaric Acid	2.61-4.5		
Caffeic Acid	3.15-12.95		
Gallic Acid	13.08- >20		
Cis-resveratrol	0.27-0.88		
Trans-resveratrol	0.71-2.50		
Catechin/epicatechin	213 and 82		
Quercetin	0.50-5.26		
Total phenolic	1621	Sangiovese	(35)
Proanthocyanidins	1217		
Caftaric acid	21.20		
Gallic Acid	15.90		
Caffeic Acid	5.20		
Ferulic acid	2.10		
Catechin	32.80		
Epicatechin	16.10		
Quercetin	3.50		
Trans-resveratrol	0.53		
Cis-resveratrol	0.11		
Total phenolic	2000	5 commercial wines from Spanish region	(68)
Gallic Acid	42.80		
Rutin	4.62		
Trans-resveratrol	1.34		
Quercetin	4.66		
Total phenolic	A 1482- B 1578- C 1489	Tinto fino	(50)*
Anthocyanidins	A 341-B 377- C 365		
Catechin	A 496- B 510- C472		
Proanthocyanidins	A 962- B 1139- C 962		
Total phenolic	A 1843- B 2137- C 2046	Cabernet Sauvignon	
Anthocyanidins	A 387- B 417- C 423		
Catechin	A 774-B 886-C 782		
Proanthocyanidins	A 1134- B 1680- C 1438		
Cis and trans-resveratrol	17.2	3 germany red wines	(56)
Cis and trans-resveratrol	5.5	13 red wines	(69)
Cis and trans-resveratrol	19.7	46 red wines	(70)
Cis and trans-resveratrol	5.6	23 red wines	(71)
Catechin	187.0	3 germany red wines	(56)
Catechin	202.0	50 languedoc red wines	(72)
Catechin	190.0	95 french red wines	(73)
Antocyanin	403	50 french red wines	(56)
Antocyanin	164	50 french red wines	(72)
Flavonols	6.5	50 french red wines	(72)
Phenolic acid	48.0	50 french red wines	(72)

* Tinto Fino and Cabernet Sauvignon graper harvest at three chosen dates A,B,C (A: was

the usual moment for harvesting; B and C: were one and two week later than the first).

TABLE 2

TOTAL PHENOLIC CONTENT IN RELATIONSHIP TO THICK OR THIN SKIN GRAPES (52)

Wines	Total flavonol content (mg/l)
Cabernet Sauvignon (1994), Chile *Thick skin grapes*	41.6
Cabernet Sauvignon (1994), France *Thick skin grapes*	24.4
Cabernet Sauvignon (1992), California *Thick skin grapes*	33.6
Cabernet Sauvignon (1990), Spain *Thick skin grapes*	10.7
Pinot Noir (1995), Chile *Thick skin grapes*	29.4
Pinot Noir (1993), France *Thick skin grapes*	9.2
Pinot Noir (1993), California *Thick skin grapes*	14.0
Pinot Noir (1990), Romania *Thick skin grapes*	7.8

TABLE 3

EFFECT OF GRAPE QUALITY ON THE RESULTING WINE POLYPHENOL CONTENT (55)

Compound	**Wine A** *Lower quality grapes* *(Cabernet Sauvignon)*	**Wine C** High quality grapes *(Cabernet Sauvignon)*
Total phenol (in gallic acid equivalent)	8.2	10.5
	91 μM	213.3 μM
Flavonols	86 μM	95.3 μM
Flavan-3 ols	73.7 μM	239.1 μM
Anthocyanins	80.7 μM	63.7 μM
Gallic acid	271.4 μM	386.7 μM
Hydroxy-cinnamates	6.7 μM	13.8 μM
FranceTotal stilbens	2.01	3.64
Antioxidant activity (ESR)		

Effect of different wine making techniques on the phenolic content of red wines

Grape processing: Netzel investigated the influence of three different grapes processing on the phenolic content of the resulting wines (56). Same grape variety was crushed and divided in three different batches: batch A had fermentation on skin, batch B was treated by mash heating and batch C was processed by mash heating and skin fermentation. Grape treatment C resulted in higher concentrations of flavonoids, stilbenes, and antioxidant capacity, then grape treatment B and A in decreasing order. Heating followed by fermentation had an great extraction influence on phenols located in the skin (anthocyanins and flavonols), the seeds (flavan-3-ols), and grape cells (resveratrols) (figure 3). No influence was noticed on the phenols produced by yeast (tyrosol) and phenolic acids located in the skin, juice and solid pulp (figure 4). These results are comparable with those published by Soleas (47). Heating treatment of crushed grapes results in fast extraction of polyphenols but without alcoholic fermentation this technique is limited in it is total extraction capacity.

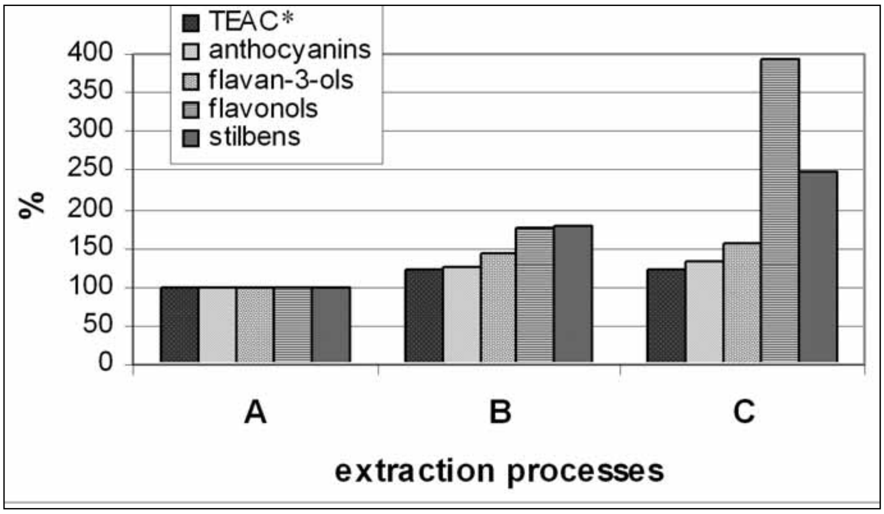


Fig. 3 - Relative changes of antioxidant capacity, and flavonoids compounds, where A= fermentation on skin, B= mash heating, C= mash heating and skin fermentation (56).

* TEAC: Trolox Equivalent Antioxidant Capacity

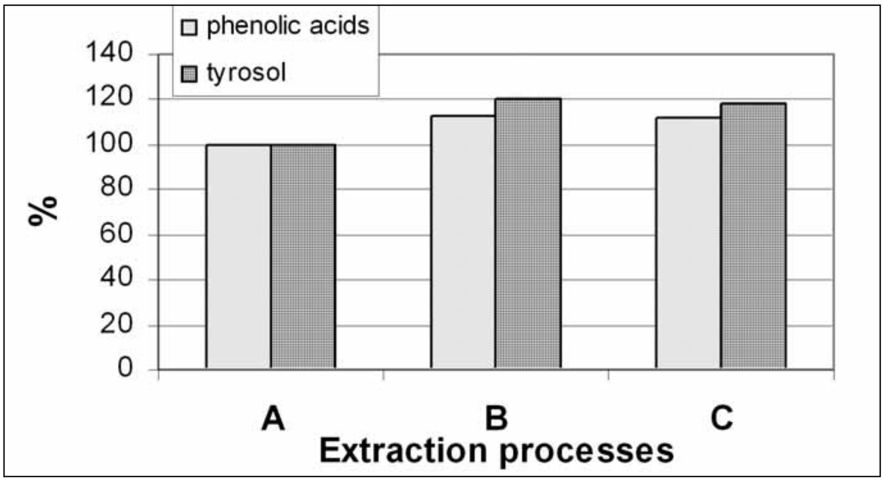


Fig. 4 - Relative changes of phenolic acids and tyrosols, where A = fermentation on skin, B = mash heating, C = mash heating and skin fermentation (56).

Oxygen supply during storage: Oxygen supply has an essential role in improving red wine color during the maturation phase (57). Castellari found that oxygenation of wines enhanced the content of large molecular weight phenolics (polymeric pigments) but decreased by about 11% the content of caffeic and ferulic acid, catechin, epicatechin and trans-resveratrol compared to the control wine (35). Thus, oxygen treatment has an negative effect on antioxidant capacity of wines threw the reduction of low molecular weight phenolics, but on the other hand stabilizes the red wine color.

Enzymatic treatment: The use of pectolytic enzymes to increase anthocyanins content in wines is a common practice in oenology. These enzymes also have beneficial impact on fermentation, pressing and clarification, increasing the content of alcohol and methanol and making their use a good candidate to future oenology biotechnology (58-61). Pardo found a higher extraction rate of polyphenols in wines under enzymatic treatment than in control wines (62).

More recently Revilla and Gonsalez-San José studied the effect of these enzymes on the content of specific phenolic compounds of wines: low-molecular-weight phenols (63). Addition of pectolytic enzymes before the inoculation of yeast to grape pomace, resulted in an increase content of phenolic aldehydes and acids. However, for flavan-3-ol monomers and polymers their concentration in wine was strongly dependant not on the enzymatic treatment but on duration of maceration.

Effect of maceration time and composition of the must: Ribereau-Gayon found that the concentration of total phenols increased with time maceration, and the phenolic extraction profile changes with time (64). The concentrations of catechins and polymers increased constantly with time maceration, whereas anthocyanins extraction increased at first and then decreased after several days of maceration (65).

In presence of whole cluster maceration the polyphenolic content of the resulting wines is higher than in wines macerated without stems, especially for the case of catechins and proanthocyanidins (table 4) (66). Same phenomenon is noticed when the maceration is done with additional seeds.

TABLE 4

CONTENT OF TOTAL POLYPHENOLS AND TOTAL CATECHINS AND PROANTHOCYANIDINS OF WINES MADE FROM WHOLE CLUSTER, DESTEMMED CLUSTER AND ADDED SEEDS (65)

Must composition for maceration	Total polyphenols (as Folin-Ciocalteu Index)	Total catechins and proanthocyanidins (mg/l)
Whole cluster	43.2	532
Destemmed cluster	35.3	393
Added seeds	77.5	1786

Conclusions

It has been demonstrated that several factors affect phenolic composition in red wine. Depending on the Vitis cultivar there are great variations in the phenolic profile. Best quality grapes produce higher content of skin-derivated phenolic classes, but the behavior is not the same for all classes of compounds. On the other hand O2 increases only high molecular weights phenols, improving at the same time wine color stability while pectolytic enzymes influence content of aldehydes and acids. Finally maceration with whole clusters or additional seeds has a positive influence on total phenolic content (67).

Depending on the pre-fermentation technique, the amount of phenolic compounds in the resulting wine can be enhanced, but these compounds don't have a uniform extraction behavior. More data in these fields are required to asses the impact each parameter on phenolic content in order to increase the amount of these substances phenolic in red wine.

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