Wine features related to safety and consumer health: an integrated perspective

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This work is dedicated to Prof. M.Carmen Polo, one of the main supporters of the research in Oenology at the Institute of Industrial Fermentations (CSIC), and whose guidance is greatly valuable in our daily work.

This review presents a global view of the current situation of the scientific knowledge about aspects of wine with possible repercussions (positive or negative) on consumer health and wine safety. The presence in wine of some potential harmful compounds such as phytosanitary products, trace metal compounds, sulfites and some toxics from microbial origin, such as ochratoxin A, ethyl carbamate and biogenic amines, is discussed. The different strategies and alternative methodologies that are being carried out to reduce or to avoid the presence of these substances in wines are also discussed. In recent years many work has been focused on establishing the scientific explanations for the positive biological effects of some wine compounds. In this review, we also examine the latest knowledge regarding wine and health, focusing in two types of compounds that have been related to the positive effects of moderate wine consumption such as phenolic compounds and bioactive peptides.

Keywords: Wine, Phytosanitary products, Trace metal compounds, Sulfur dioxide, Ochratoxin A, Ethyl carbamate, Biogenic amines, Phenolic compounds, Peptides

INTRODUCTION

The intense changes in society's life style over the past few years have had significant effects on the basic concepts of our eating habits. Nowadays, we can go further than the traditional idea of what should be a 'suitable diet' in the sense that it should provide the sufficient nutrients to ensure individuals' survival, satisfy metabolic requirements and pleasurably appease the sensation of hunger and wellbeing. Today, it is also necessary to comply with aspects of health and safety, emphasizing the potential of foods from a health perspective, to increase wellbeing and to reduce the risk of illnesses.

In an attempt to guarantee food safety for the consumer, several organizations such as the Food and Agriculture Organisation (FAO) of the United Nations and the World Health Organisation (WHO), among others, assess the health quality of foods in general, and also of wine. However, for aspects related to wine safety there is also another authority, the International Organisation of Vine and Wine (OIV), which, among other objectives, helps to protect consumer's health, improving and guiding research on health aspects and spreading out the results of this research to healthcare professionals. Within this Organisation, which encloses several Commissions and Subcomissions, the Safety and Health Commission, is included. This group of experts is mainly focused on food safety, wine consumption, nutrition and health.

In recent years, the topic 'wine and health' has aroused much interest but also considerable controversy. This is support by the large number of studies and scientific contributions that has been carried out within this area. In this review, we have summarised the main contributions of current knowledge about aspects of wine and its components that could affect safety and consumer's health. We do not specially focus

on alcohol, a wine ingredient with evident and well-known toxic effects, but instead on wine as a food, that can have beneficial effects on the health of healthy adult consumers when it is drunk in moderation. Nevertheless, wine may contain certain compounds that decrease the wholesomeness of wine such as chemical preservatives (sulphites), neurotoxins (ochratoxin A), potential carcinogens (ethyl carbamate) and allergens (biogenic amines), amongst others. Therefore, besides the need to examine the main causes of wine spoilage that may affect human health, this article also discusses the strategies followed to eliminate or minimise wine contamination. The latest studies about those compounds involved in positive health aspects of wine are also reviewed.

COMPOUNDS PRESENT IN WINE WITH POTENTIALLY NEGATIVE HEALTH EFFECTS. METHODS FOR THEIR CONTROL AND PREVENTION

Over the past few years, numerous research studies have focused on establishing the components of wine that can negatively affect the health of moderate wine drinkers, with the ultimate objective of searching for solutions to decrease or to avoid their presence in wines. The compounds that can damage wine's health quality include those derived from the grape, which can appear as contaminants (i.e. pesticides and other phytosanitary products, trace metal compounds, additives such as SO_2, which is added during different steps of the wine production process), and those produced by different microorganisms during winemaking including, ochratoxin A, ethyl carbamate and biogenic amines.

Phytosanitary products

Nowadays, a wide range of insecticides, fungicides and herbicides are used in grape production. In particular, grapes are especially vulnerable to moulds, which leads to the widespread use of antifungal agents. In Mediterrenean countries, the principal parasites of vine are the grape moth (*Lobesia botrana*), downy mildew (*Plasmopora viticola*), powdery mildew (*Unicinula necator*) and gray mold (*Botrytis cinerea).* The latter, if conditions are appropriate, may attack bunches of grapes damaging the grape, by synthesizing the enzymes required to breakdown the cell walls of the skin and penetrate in the flesh. To control these parasites, insecticides and fungicides are commonly used. In practise, to tackle this and other alterations, it is much more effective to use preventive treatments in order to maintain the vine whole and improve the quality of the grape. Although the correct use of pesticides does not cause environmental problems or public health concerns, if an inappropriate and abusive pesticide treatment is carried out, undesirable residues may remain on the grapes after harvest. As a result, they may be transferred into the wine. A more detailed review in this area was published by Cabras and Angioni (2000).

With the aim to improve hygienic and sanitary characteristics of wines, the effect of some wine-making practices on the concentration and disappearance of pesticide residues during wine production has been evaluated (Cabras et al., 1995, Navarro et al., 1997, Soleas and Goldberg, 2000). Recently, some clarification and filtration processes have proved to be effective against four commonly used fungicides (cypronidil, fludioxonil, pyrimethanil, and quinoxyfen) for the control of cryotogramic vineyard diseases (Fernández et al., 2005). The most common clarifying substances for wine are bentonite, charcoal, gelatin, polyvinylpyrrolidone, potassium caseinate, and coloidal silicon dioxide. While most of them show limited ability to decrease pesticide

residues, charcoal is especially recommended when the amount of residues is low, allowing the complete elimination of most pesticides. Its effectiveness generally decreases as the pesticide water solubility increases; therefore, pesticides highly soluble in water, such as dichlorvos and dimethoate, do not show appreciable changes (Cabras et al., 1995).

The activity of yeast and lactic acid bacteria involved in alcoholic and malolactic fermentation respectively, can be affected by the presence of pesticide residues in musts and wines (Cabras and Angioni, 2000). Nevertheless, while it has been shown that some wine yeasts are able to reduce the pesticide content, either by degradation or by adsorption (Cabras and Angioni, 2000), lactic acid bacteria do not posses this ability.

Although maximum residue limits for most pesticides in wine have not been set, several countries have established guidelines for the correct use of pesticides and for the maximum residual limits for the treatment of vines and grapes used in winemaking (Flamini and Panighel, 2006). Because the vinification process decreases the levels of pesticides, their content in wines is significantly lower than in grapes. Therefore, methods to detect pesticide residues must be very precise and sensitive. Pesticides have been analyzed in wines mostly by gas chromatography (GC) (Jiménez et al., 2001, Hyötyläinen et al., 2004) and high-performance liquid chromatography (HPLC) (Nozal et al., 2005; Braga et al., 2007) and more recently, by capillary electrophoresis (CE) (Molina-Mayo et al., 2006). Mass spectrometry represents an advantageous alternative for the simultaneous determination of different pesticides in wine. Recently, an interesting review about the applications of mass spectrometry for the determination of pesticides and other contaminants in grapes and wines has been reported (Flamini and Panighel, 2006).

One aspect that deserves special attention is that phytosanitary treatments could be reduced if the plants own defence mechanisms come into play. Phytoalexins are antimicrobial compounds produced by plants, such as grapevine, in response to biotic and abiotic stress factors and have received a lot of attention over the last decade (Jeandet et al., 1997; Jeandet et al., 2002). These compounds can be released from plants as a response to infections by pathogens and hinder their development**.** They are often synthesized within a few hours after stress exposure. They accumulate in the plant reaching a maximum content between 2 and 3 days after the induction. Therefore, stimulating the production of grapevine phytoalexins could be a strategy to limit the use of pesticides in vineyards. In the *Vitaceae* family, phytoalexins have been well characterized. They constitute a rather restricted group of molecules belonging to the stilbene family (for a review see Jeandet et al., 2002). Resveratrol (3,5,4'-trihydroxystilbene) is the major phytoalexin produced in grapevine as a general response to different agents: fungal attack, leaf UV-C irradiation, heavy metal exposure, and ozone treatments. Other biosynthetically related compounds that have been found in grapevine as a result of stress conditions are viniferins and pterostilbene (3,5-dimethoxy-4'-hydroxy stilbene) (Jeandet et al., 1997). A novel approach in the actual research on phytoalexins is the development of synthetic phytoalexins, similar to the natural ones, that can be used for plant disease control. Stilbene phytoalexines are formed on the phenylalanine/polymalonate pathway and the last step in their biosynthesis is catalyzed by the enzyme stilbene synthase (STS) (Jeandet et al., 2002). Therefore, another valuable scientific strategy that is being performed is focused on the transfer of STS gene from grapevine to numerous plants, with the objective of increasing their tolerance to pathogenic microorganisms. Following this strategy, it has been possible to enhance the resistance of grapevine rootstock 41B to the infection of

Botrytis cinerea. In addition to plants, the gene has been successfully transferred to yeast and mammalian cells (Zhang et al., 2006).

Trace metal compounds

Although trace elements are essential for humans, many of them (e.g. As, Sb, Bi, Pb, Sn, Se, Te) have unknown biological function and are regarded as toxic because they accumulate in the human organism. In fact, at slightly higher levels than when found in living beings, they can have toxic effects (Baluja-Santos and Gonzalez Portal, 1992).

It is well known that some trace elements play a relevant role in winemaking, for example Zn is essential at low concentrations, for the correct development of alcoholic fermentation, while Cu, Fe, and Mn have organoleptic effects at increased levels, contributing to the haze and taste of wines (Riganakos and Velssitas, 2003). Excessive amounts of iron (10-20 mg/L, or more) can cause stabilization problems in wines, since it may be oxidized to a ferric form causing precipitation of pigmented materials ('Blue haze') or with orthophosphate ions ('White haze').

Nevertheless, as Ajtony et al., (2008) have stated, the concern about human exposure to trace metals in beverages and food products, including wines, has started to attract considerable attention, since the consumption of wines, especially in fairly large volumes, may significantly contribute to the daily dietary trace element intake by humans.

The primary natural mineral content of wines is determined by many factors such as type of soil, variety of grape, weather conditions, pollution and viticultural practices. In fact, the regionally varying trace metal content of wines can also be used for source

identification purposes (Pyrzynska, 2004). The secondary mineral contamination is derived from the use of pesticides and fertilizers or acquired during winemaking (in spite of the overall reduction in mineral content during this process) (Baluja-Santos and Gonzalez Portal, 1992). It has been shown for example, that while the origin of Cu in wines is associated with copper-based vineyard sprays, the content of As, Cd, and Pb reflects the differences in grape variety, environmental factors (e.g., soil, climate), and winemaking technology (Pyrzynska, 2004).

Sodium arsenite is employed in viticulture as fungicide against a grapevine necrotic disease known as *Eutypa* dieback, caused by the fungus *Eutypa lata*. Arsenic is a suspected carcinogen and little is known about its chronic sub-lethal effects. The concentration of As in wines mainly depends on factors such as soil composition, grape variety, climatic conditions, use of pesticides, winemaking technology and storage conditions (Flamini and Panighel, 2005). The OIV has established the maximum limit of As in wine at 200 pg/µl, but in general, the presence of only a few pg/µl of As in uncontaminated wines is accepted (Baluja-Santos and Gonzalez Portal, 1992; Pedersen et al., 1994; Bruno et al., 1994).

In the case of lead, its presence in wine could be the consequence of the airbone particulate matter of atmosphere on grapes, and/or by the grapevine intakes from ground water and soil. The metal may also be released from bronze tanks, taps, pumps and tubing containers use in winemaking (Flamini and Panighel, 2005). Lead adversely affects multiple enzyme systems of the body, as any ligand with sulfhydryl groups can be vulnerable. The best-known effect is that of the production of heme (Bornet and Teissedre, 2008) . Detection of lead in wine is usually focused on the determination of the total lead Pb^{2+}. In wine the structurally complex pectic polysaccharide rhamnogalacturonan II (RG-II), an anionic biomolecule released from the grape during

winemaking, can form coordination complexes with specific cations such as lead (Flamini and Panighel, 2005). This pectic polysaccharide could reduce intestinal absorption and tissue retention of Pb as has been reported in rats, with a potential beneficial effect by minimising toxicity (Tahiri et al., 2000; Tairi et al., 2002).

The toxic effects of cadmium are due to the inactivation of enzymes containing sulphydryl groups and the uncoupling of oxidative phosphorylation in mitochondria. Cadmium may also compete with other metals such as zinc and selenium for inclusion into metallo-enzymes and it may compete with calcium for binding sites on regulatory proteins such as calmodulin (Bornet and Teissedre, 2008). The OIV establishes the maximum concentrations of lead and cadmium in wine at 150 µg/L and 10 µg/L, respectively.

Taking into consideration the importance of these elements regarding their potential toxicity and technological features, it is worth noticing the great amount of scientific work that has been focused in developing adequate analytical tools that can offer enough selectivity, sensitivity and robustness for the analysis of these compounds in wines. As consequence, there are many analytical techniques for quantification trace metal in wines, such as ion chromatography (IC), stripping potentiometry, inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), flame atomic absorption spectrometry and graphite furnace atomic absorption spectrometry (GFAAS). All of them have been extensively reviewed elsewhere (Pyrzynska, 2004; Flamini and Panighel, 2006; Ajtony et al., 2008).

The multiple sources involved in the content of metal trace elements in wines make difficult the establishment of appropriate strategies to prevent or to avoid their occurrence in wines. Obviously, an adequate soil for each grape variety and environmental conditions, the regulation in the use of fertilisers, fungicides and others

pesticides or an adequate winemaking equipment could greatly reduce the amount of these elements in wines. Recently, La Pera et al., (2008) in a study performed over two vintages (2003, 2004) have shown that treatments with the fungicides mancozeb, zoxamine and copper oxychloride significantly increased the levels of Mn, Zn, Cu, Pb and Cd of wines, grapes, marcs and grape stalks.

Some strategies that could be effective for the control of trace heavy metals imply the use of some polymers from natural sources. Chitin, chitosan and other derivatives are non-toxic biodegradable polymers that can remove metals and organic contaminants from food products (Bornet and Teissedre, 2005). Chitin exists widely in the cell walls of fungi, moulds and yeasts but also in the exoskeletons of some invertebrates such as molluscs, insects, etc. Chitosan is found only in a few species of fungi. Chitin and chitosan consist of 2-acetamido-2-deoxy-β-D-glucose and 2-amido-2-deoxy- β-D-glucose as repeating units, respectively. Bornet and Teissedre (2008) showed that the addition of chitosan, chitin, chitin-glucan and chitin-glucan hydrolysate of fungal origin (until 2 g/L) to wine, can reduce the amount of Fe by 32-91%, Cd by 11-57% , and Pb by 33-84 %, depending on the type of wine.

Furthermore, based on the parietal adsorption activity of yeasts (Caridi, 2007), the use of inactive yeast or yeast derivatives (walls, hulls, etc.) in wines, could also be considered in the future as a control mechanism for trace metal compounds to improve wine safety.

Sulphur dioxide

One compound that should be considered in relation to the health quality of wines, is sulfur dioxide (SO_2). Because of the abundance of sugars, nitrogen

compounds, vitamins, mineral and salts, grape must is an ideal medium for microbial growth. SO_2 has been used since the times of Romans for disinfection and cleaning of wine cellars. Although its use as preservative in wines has been known for centuries, it has been only recently, when its use in prefermentation operations during winemaking has been spreading out. Most of the scientific knowledge about the use of sulphur dioxide in oenology has been acquired over the last decades. In wines, this compound has multiple and diverse properties. For example, it is an *antiseptic* agent against yeasts and bacteria. The *antioxidant* properties, are well established since it consumes oxygen and acts against non-enzymatic and enzymatic oxidation of wines. Moreover it behaves as a *solvent,* activating the extraction from the solid parts of the grape, (skins, seeds, stems). Finally, since it favours the static settling of wines, it can be considering as a *clarification agent*. These and other properties, make this compound widely used during the different steps of winemaking and storage.

During winemaking, SO_2 is mainly used on three occasions. Firstly, in the grapes or must during the prefermentation step, with the prime objective of preventing its oxidation; later, to inhibit microbial growth that can alter the wines, once the fermentation processes have finished and before the ageing or storage steps, and, finally, just before bottling, to stabilise the wines and prevent any alteration or accident in the bottles.

Today, the addition of SO_2, is therefore, an essential treatment in winemaking technology. However, the use of this additive is strictly controlled, since high doses can cause organoleptic alterations in the final product (undesirable aromas from sulphurous gas or from the reduction products, hydrosulphate and mercaptanes) and, especially, owing to the risks for human health derived of its consumption.

Various studies have revealed that besides their multiple properties, sulphites may induce relevant adverse effects after their ingestion (Taylor et al., 1986). Moreover, it has been shown that considerable percentage of consumers, show intolerance or high sensitivity to sulphites. This risk is higher for asthmatics and children. It is estimated that almost 1% of the population and 5% of the asthmatics are concerned. Although sulphites do not cause a true allergic reaction, sulphite-sensitive people may experience some similar responses. The symptoms may develop quickly and can include flushed face; rash, red and itchy skin; headaches; eye, face, lip or throat swelling; difficulty for breathing, speaking or swallowing; anxiety, distress, faintness, weakness; cramps, diarrhoea, vomiting, among others.

The upper limit of sulfites permitted by the OIV ranges from 150 to 400 mg/l of total SO_2, depending on the type of wine and its content in reducing matter. A first step to increase the food safety has been done by the EU through the approval of the legislation for regulation the use of sulphites as preservatives. Since then, directives 2000/13/EC, 2003/89/EC and 2007/68/EC request the systematic labelling of allergens or similar (annexe III bis from directive 2000/13/EC) incorporated in food products. In the same way, since November 25th of 2005, is mandatory to declare the presence of sulphites in wines and other foodstuffs when the concentration exceeds 10 mg/L or 10 mg/kg. In addition, directives 95/2/EC and 2006/52/EC precise the maximum sulphite concentration allowed in different foodstuffs (included wine) within the EU.

These legislative rules, together with consumer demands, have attracted the interest of scientific research on the study of other wine preservatives, harmless to health that can replace or reduce the amount of SO_2 in wines. One example is the production of ‘ecological wines’, which in recent years have gained considerable interest. They are produced by environmentally friendly methods, from integrated or

sustainable manufacture methods. During the production of these wines, only a series of strict oenological practises are permitted and no additive apart from SO_2 is possible. The final wine must have as low amount of sulphur dioxide as possible and must not, under any circumstance, exceed very strict limits (from 70 to 100 mg/l depending on the type of wine (EC Directive 2092/91).

Physical treatments, such as modified atmosphere packaging, control atmosphere storage, ozone, and other alternative non-conventional gas treatments have been applied to table grapes in order to prolong shelf life and storage, therefore reducing the SO_2 doses necessary in the harvest (Artés-Hernández et al., 2003; Artés-Hernández et al., 2006).

For wines, other methods for sulfites removal such as adsorption through the use of anion and cation exchangers (Brown, 1991) or the use of a membrane reactor in conjunction with non-diffusible oxidizing agents for sulfite oxidation (Seifter et al., 1994) have been tested. Although both methods can reduce the sulfite content of wines below 10 ppm, the lack of selectivity in their adsorption mechanisms can greatly decrease important flavor compounds negatively affecting wine quality. Another original alternative for reducing sulfite contents in wine is the use of wheatgrass (*Triticum aestivum*) chloroplasts for the oxidation of sulfites to innocuous sulfates. It has been shown that a crude chloroplasts preparation at a concentration as low as 5mg/mL, is able to reduce sulfites in commercial white wines from 150 ppm to 7.5 ppm, as well as up to 93 % of the initial sulfite content in red wines within 45 minutes (Lin and Georgiou, 2004). Although this simple bio-catalytic process seems to be highly effective, inexpensive and largely valuable for the winemaking industry, appropriate sensory analysis would be necessary to evaluate the quality of these wines.

The effects of electrochemical treatments and pulsed electric fields on the growth and metabolism of wine yeast have been studied (Ranalli et al., 2002) and applied for controlling grape must fermentation in winemaking (Lustrato et al., 2003). These treatments could be an alternative to the use of SO_2 since they are capable of destroying microorganisms and some enzymes while maintaining the characteristics of food products (Benicho et al., 2002). Nevertheless, more studies should be necessary to evaluate the potential use of these technologies in winemaking.

Regarding other chemical alternatives to the use of sulphites, the use of dimethyldicarbonate (DMDC) has been recently described. This compound, also known as E242, can inhibit the development of spoilage and fermentative yeasts, allowing to reduce the dose of SO_2 in some types of wines (Threlfall and Morris, 2002; Divol et al., 2005). It also acts on bacteria and mould development, inactivating some vital enzymes for these microorganisms, therefore causing their death. DMDC is also currently used for cold sterilization of different types of drinks.

Other alternatives focused on 'natural antimicrobial agents' have been developed to reduce the use of sulfite in wines. Among them, lysozyme (EC 3.2.1.17) has been recently introduced into the wine industry and offers important advantages for controlling malolactic fermentation in wine (Bartowsky, 2003; Pilatte, et al., 2000). Lysozyme (1,4-β-*N*-acetylmuramidase) has the ability to cleave the β-1,4-glycosidic bonds present in Gram-positive bacteria. It has no effect on yeasts, is not affected by alcohol and is active in the pH range of the winemaking process. As an organoleptically neutral alternative, lysozyme can be beneficial to further reduce SO_2 levels during winemaking. As wine pH increases, the antimicrobial activity of lysozyme increases as well, which makes it attractive for preventing spoilage in wines with high pH (Gao et al., 2002; Delfini et al., 2004). Despite lysozyme was firstly authorized as an additive in

winemaking due to the high costs of its application, its use has been largely reduced. Another aspect that deserve consideration, is the IgE-mediated immune reactions that lysozyme can cause in some individuals (Mine and Zhang, 2002). Therefore its presence in food products, including wine, can cause some health concern.

Currently, it is well known that other polypeptidic compounds, called bacteriocins, can also produce an inhibitory effect on the development of lactic acid bacteria. These products have received great attention in the manufacture of dairy products, since they are especially used as additives during cheese ripening (Martínez-Cuesta et al., 2003). Because of the lack of effects of bacteriocins on wine organoleptic properties and the absence of toxic effects, some studies to evaluate their use in the control of malolactic fermentation during winemaking have been conducted (Navarro et al., 2000; Bauer et al., 2003, 2005). Generally, bacteriocins can be introduced into the wine by direct addition of the peptide (normally in its purified form), by adding a supernatant of a bacterial culture that contains a crude extract of the peptide, or by inoculation of a starter culture of lactic acid bacteria. It has also been shown that after application of gene technology to transform laboratory strains of *S. cerevisiae* with the pediocin and leucocin structural genes, *pedA* and *lcaB* respectively, the transformed yeasts were able to produce biologically active bacteriocins, although in low levels (Schoeman et al., 1999). Despite the use of bacteriocins can be appropriate for inhibiting the growth of spoilage bacteria in wines, up to date, nisin is the only bacteriocin that can be obtained commercially. Although it has been shown to be effective (Radler et al., 1990a,b; Rojo-Bezares et al., 2007), its use has not been authorized in winemaking.

Another strategy that has been proposed to reduce the use of SO_2 in oenology, is by using antimicrobial metabolites such as hydrogen peroxide (Du Toit et al., 2000).

Glucose oxidase (GOX) from *Aspergillus niger*, which has GRAS status, is of considerable industrial importance. GOX metabolises glucose into gluconic acid and the antimicrobial compound that results from this conversion is hydrogen peroxide (H_2O_2). H_2O_2 has been shown to be active against both Gram-positive and Gram-negative bacteria. The *gox* gene was expressed in *S. cerevisiae* and the GOX-producing yeast transformant inhibited the growth of both acetic and lactic acid bacteria (Malherbe et al., 2003).

Currently there are increasing evidences of the potential application of some eukaryotic antimicrobial proteins and peptides as food preservatives (Rydlo et al., 2006). Among others, antimicrobial peptides derived from food proteins present clear advantages to be used in food preservation (Pellegrini, 2003). Milk is a particularly interesting source of antimicrobial peptides that can be released after digestion with protease. Among them, lactoferrin (LF) is a multifunctional iron glycoprotein that exhibits a diverse range of biological effects, including antimicrobial, antiviral, antioxidant, and immunomodulatory activities (Tomita et al., 2002). Recently, its antimicrobial properties against phytopathogen molds has also been described (Muñoz and Marcos, 2006). Several strategies based on the use of hydrolizated LF have been also investigated to control microbial growth of *Saccharomyces cerevisiae* and other spoilage non *Saccharomyces* wine yeasts (*Cryptococcus albidus*, *Dekkera bruxellensis*, *Pichia membranifaciens*, *Zygosaccharomyces bailii* and *Zygosaccharomyces bisporu)* (Enrique et al., 2007). Further studies would be required to gain more knowledge about the mode of action of these antimicrobial agents in wines and their consequences for wine quality.

Recently, special attention has been paid to the potential of some natural constituents of grapes and wines, such as phenolic compounds, as an alternative to

sulphites (García-Ruiz, et al., 2008a). It has been suggested that phenolic compounds can behave as activators or inhibitors of bacterial growth depending on their chemical structure (substitutions in the phenolic ring) and concentration (Reguant et al., 2000; Vivas et al., 1997). However, data are scarce since research has mainly been focused on studying the effect of hydroxycinnamic and hydroxybenzoic acids and some flavonols (Stead, 1993; Rozes and Peres, 1998; Campos et al., 2003; Alberto et al., 2001). In a recent study, the evaluation of the dual antioxidant and antibacterial activity of 21 phenolic compounds mainly present in *Vitis Vinifera L.* belonging to different groups (hydroxybenzoic acids and their derivatives, hydroxycinnamic acids, phenolic alcohols and other related compounds, flavonols, anthocyanins and stilbenes) was examined (García-Ruiz et al. 2008b). Through the use of structure-activity relationships, the antimicrobial and antioxidant properties of some of these wine phenolics was demonstrated, confirming the potential use of these compounds as an alternative to sulphites in winemaking. Nevertheless, more studies would be necessary to evaluate other phenolic structures present in wines and to elucidate the mechanism of action implicated on microbial inactivation.

Compounds resulting from microbial metabolism

Winemaking cannot take place without the action of microorganisms, mainly yeasts, and also lactic acid bacteria. Yeasts are essential to obtain wine, and lactic acid bacteria to make an important contribution to its organoleptic quality. However, the winemaking process includes multiple stages at which spoilage microoorganisms, mainly some strains and species of moulds, yeasts and lactic acid bacteria can develop and alter the quality and hygienic status of the wine, affecting the food safety for the

consumers. Some interesting examples include the production of ochratoxin A, ethyl carbamate and biogenic amines, that will be discuss as follows.

Ochratoxin A

Ochratoxin A (OTA) is one of the most common naturally occurring mycotoxins, which is receiving increasing attention due to its toxic effects and high incidence in a wide range of food products. The IUPAC developed formula of OTA is 1-phenylalanina-*N*-[(5-chloro-3,4-dihydro-8-hidroxy-3-methyl-1-oxo-1H-2-benzopyran-7-yl)carbonyl]-isocoumarin (Ringot et al., 2006) (**Figure 1**). OTA is a mycotoxin considered to be a possible carcinogen (Class 2B) in humans (IARC, 1993) and it has been shown to be nephrotoxic, teratogenic, hepatotoxic and inmunotoxic in animals, and possibly in humans (Zimmerli and Dick, 1996). It has also been associated with the Balkan Endemic Nephropathy (BEN) and with the development of urinary tract tumors. Unfortunately, the presence of this compound in blood from healthy humans confirms a continuous and widespread exposure (Creppy, 2002).

Since 1970 the occurrence of OTA in foods has been investigated and its presence has been confirmed in different food products such as cereals, coffee, some sauces such as soya sauce, cocoa, dried fruits, meat and beer (JECFA, 2001). In the European diet, wine and especially red wine, has been identified as the second major source of human exposure to OTA, following cereals. The occurrence of OTA in wine and grape has been reported in different countries, but higher levels of OTA were found in the southern regions of Europe than in northern regions (Zimmerli and Dick, 1996; Visconti et al., 1999; Otteneder and Majerus, 2000; López de Cerain et al., 2002; Bacaloni et al., 2005). Consequently, the European Commission has considered that is

necessary to impose regulatory limits establishing 2 μg/L as the maximum level of OTA in wine and grape products (EC Nº 123/2005).

OTA is produced by the secondary metabolism of several *Aspergillus* and *Penicillium* species, mainly by *Aspergillus ochraceous* and *Penicillium verrucosum* (Chulze et al., 2006). Recently, black *Aspergillus* species (section *Nigri*), including *Aspergillus cabonarius, Aspergillus aculeatus* and *Aspergillus japonicus,* have been described as the main source for OTA contamination in grapes (Da Rocha Rosa et al. 2002; Battiliani et al., 2003; Serra et al., 2003; Bellí et al., 2004; Leong et al., 2006a). Researchers agreed in considering *A. carbonarius* as the main causal agent of OTA presence in grapes in Europe and Israel (Battliani et al., 2006; Belli et al., 2006). Molecular methods (for an overview see Abarca et al., 2004) have led to an important progress in the identification of the fungi species responsible for OTA contamination in grapes and wine.

Improvements in viticultural and winemaking practices are required to reduce OTA in wines. The great amount of work performed on this topic, has allowed to gain knowledge about the origin and mechanisms to explain the presence of OTA in wines and in the development of prevention and control strategies to avoid its occurrence. Recently with the aim to limit the presence of OTA in vine-derived products, the OIV has adopted a 'Good Viticultural and Winemaking Practices Code' based on recommendations and specific interventions for both harvest and cellars (OIV, 2005). The main measures considered by the OIV Code are summarized in **Table 1**.

Factors affecting the presence of ochratoxin A in wines have been widely reviewed (Blesa et al., 2006; Mateo et al., 2007). Different practices in grape cultivation such as the type of cultivars, and in winemaking, including the storage conditions of the harvested grapes and the length of the storage, can influence the accumulation of

mycotoxins (Zimmerli and Dick, 1996). Some grape varieties may exhibit a greater susceptibility than others to *Aspergillus* bunch rots. However, once damage is set, there are no inherent differences in resistance to mould growth between different grape varieties. The substantial processing that grapes undergo during wine production is one of the major factors that influence OTA levels in wine (Leong et al., 2006b).

Regarding preventive and corrective actions to reduce OTA content in wines, it should be taken into account that a good management of the vineyard guarantees a relevant decrease in OTA content at harvesting. A primary focus for continuing research in this subject is the development of vineyard management strategies to minimize the risk of OTA development in grapes and vineyard by reducing the incidence of black *Aspergillus* spp., in soils and grape brunches. One approach in this direction is the introduction of varieties that are more resistant to splitting and hence less susceptible to *Aspergillus* bunch rots. Further research should be conducted in order to examine the genetic relatedness of black *Aspergillus* isolates from various viticultural regions. Although additional OTA is not synthesised during winemaking, a careful management during wine production including corrective actions, would contribute to minimise OTA levels in wine. Data recently presented indicate that phenolic antioxidant compounds suppress OTA production in several important ochratoxigenic *Aspergillus* species (Palumbo et al., 2007). Whether searching for natural strategies for the reduction of OTA contaminations or the study of biosynthetic and regulatory genes involved in both OTA production and stress response in ochratoxigenic Aspergilli, can be useful tools for controlling OTA in wines.

During both red and white winemaking, the removal of precipitated yeast cells and other sediments after fermentation results in further reductions in OTA content. Binding and precipitation of OTA may be enhanced through the addition of fining

agents. The influence of some oenological practices on OTA concentration in wine has been evaluated (Gambuti et al., 2005). Microfiltration (0.45 μm) and the use of carbon are two useful treatments for wine decontamination, but the use of carbon results in the adsorption of several aroma compounds (Olivares-Marín et al., 2008). The feasibility of using selected adsorbents (bentonites, modified bentonites and chitosan) to reduce OTA levels in red wine has been recently investigated (Kurtbay et al., 2008). Quitine from yeast walls (2-acetamido-2-deoxi-B-D-glucose) has been shown to reduce OTA levels in wine (Bornet and Teissedre, 2008). In addition, yeast mannoproteins also play an important role for the removal of OTA because of their binding capacity (Bejaoui et al., 2004).

Subsequent operations during winemaking also reduce the OTA content of wine; This reduction has been shown to depend on several factors. In the case of red winemaking, the fermentation step reduces OTA content both *in vitro* and in true scale experiments; the efficacy of fermentations, both alcoholic and malolactic, in reducing OTA content is strictly dependent on the yeast and bacterial strains involved, and on the level of contamination (Mateo et al., 2007). In white winemaking, OTA content always decreases and the rate of decrease depends on the choices for yeasts during alcoholic fermentation and on the processing aids added for clarification.

Ethyl carbamate

Ethyl carbamate, also known as urethane, has been used as antiseptic for many years in the alcoholic drink industry. At the beginning of the Seventies, a scientific article reported for the first time that this compound could have potentially carcinogenic properties in laboratory animals when present at high concentrations (Canas et al.,

1989). Since then, its use in food products is forbidden and considerable research regarding the mechanisms implicated in its formation in fermented and non-fermented foods, has gained interest. From the data obtained in experimental animals, it has been estimated that the daily dose for humans with no harmful effect is 0.3 ng/kg of body weight/day, which corresponds to 0.021 μg/day for a person of approximately 70 kg.

The formation of ethyl carbamate in wine is mainly attributed to the metabolism of the yeasts responsible for alcoholic fermentation. These microorganisms can sometimes produce a large amount of an intermediate metabolite, urea, that although in itself is not harmful to health, if the conditions are appropriate (high temperature, presence of ethanol and low pH), it can be transformed by a chemical reaction into ethyl carbamate during wine storage (Monteiro and Bisson, 1991). But also, it has been demonstrated that during malolactic fermentation, lactic acid bacteria can produce other precursors of ethyl carbamate such as citruline and carbamyl phosphate, which, in the aforementioned conditions can result in the production of this compound (Liu et al., 1995; Liu et al., 1996; Arena et al., 1999). In both cases, the initial substrate is arginine, one of the major amino acids present in grape must and wine (Lehtonen, 1996; Moreno-Arribas et al., 1998).

The formation of ethyl carbamate by lactic acid bacteria, has probably been the line of research most followed in recent years in this field, especially in an attempt to reduce its concentration in wines. Certain wine-associated lactic acid bacteria have the ability to utilise arginine; these include strains of *Oenococcus oeni* and heterofermentative lactic acid bacteria, such as *Lactobacillus brevis, L. buchneri, L. hilgardii*. Homofermentative lactic acid bacteria (e.g. *L. delbrueckii, L. plantarum*) and pediococci do not catabolise arginine. Now, both, the enzymatic route involved (i.e. the arginine deiminase pathway) and the genes that encode the corresponding enzymes of

oenological lactic acid bacteria species are known (Tonon et al., 2001). From the research performed, Mira de Orduña et al. (2001) concluded that the risk of citrulline formation by malolactic bacteria in wines with high residual arginine concentrations can be reduced by carrying out malolactic fermentation with pure oenococcal cultures and by precisely establishing complete malolactic conversion, which must be followed by inhibition of bacterial activity. Also, in this case, scientific results indicate the need for caution in the selection of starter cultures for MLF in wine, since citrulline formation from arginine degradation could result in ethyl carbamate production, even at normal temperatures, during prolonged storage**.** In addition, spontaneous malolactic fermentation by undefined strains should be avoided, as this may lead to formation of ethyl carbamate precursors.

Nowadays vine growers and winemakers have sufficient knowledge to prevent or reduce the formation of ethyl carbamate during the production process. The strategies for controlling the presence of this compound in wines are focused on the three factors involved in its production: vine growing and wine production practises that could increase the concentration of arginine in the grape; to control the winemaking process, especially the alcoholic and malolactic fermentations, and finally, the ageing and maturation of the wine. On the other hand, legislation authorises the use of an enological additive, the enzyme urease, in wines with high urea levels. The urease used is isolated from *Lactobacillus fermentum*. This enzyme has been effective on urea at doses of 50 mg/L in red wines and at doses of 25 mg/L in white wines (Bertrand, 1997).

A systematic study carried out to examine the ethyl carbamate levels of Spanish red wines from different grape varieties (Uthurry et al., 2004), showed that typical Spanish red wines had concentrations of ethyl carbamate below 30 ppb. During the alcoholic fermentation of the corresponding musts no ethyl carbamate was formed, even

in the presence of nitrogenous substrates, and low concentrations were recorded (≤ 1.3 ppb). Generally, regarding urea levels founded at the end of the alcoholic fermentation it was concluded that small quantities of ethyl carbamate would be formed under the normal storage conditions of wines (Uthurry et al., 2007) .

Biogenic amines

Amines are widely occurring compounds in nature, containing one or more organic substituent bonded to a nitrogen atom. Biogenic amines are low molecular weight compounds, derived from aromatic or cationic amino acids and all of them have one or more positive charge and a hydrophobic skeleton. Depending on their chemical structure they can be classified in aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) (**Figure 2**). They are endogenous of plants but can also be found in fresh fruit and vegetables. However, amines are mainly formed in foods by fermentative processes and during ageing and storage. Recently, knowledge concerning the presence of amines in wines has been reviewed (Ferreira and Pinho, 2006; Ancín-Azpilicueta et al., 2008).

Problems related to biogenic amine formation affect numerous fermented food products consumed more frequently than wine, such as cheese, beer, some fermented sausages and meat products among others (Fernándes-García et al., 1999; Izquierdo-Pulido et al., 2000; Kaniou et al., 2001), which have higher levels of these compounds. However, in alcoholic drinks, especially in wine, biogenic amines have received more attention, because ethanol and acetaldehyde can increase their effects on health by directly or indirectly inhibiting the enzymes responsible for the detoxification of these

compounds in the intestinal tract (Maynard and Schenker, 1996). Although there are differences in individual susceptibility to intoxication by biogenic amines, several pharmacological reactions can take place after excess intake of these compounds. The most well-known reactions are those caused by histamine. More specifically, some histamine–induced symptoms include rash, edema, headaches, hypotension, vomiting, palpitations, diarrhoea and heart problems. Other amines, such as tyramine and phenylethylamine, can cause hypertension and other symptoms associated with vasoconstriction due to the release of noradrenaline (especially cerebral haemorrhages and migraine). Although putrescine and cadaverine are not themselves toxic, they can increase the toxicity of histamine, tyramine and phenylethylamine, since they interfere in detoxification reactions.

Some polyamines, such as putrescine, may already be present in grape berries (Halász et al., 1994; Bover-Cid et al., 2006; Del Prete et al., 2008) or be produced during fermentation processes, ageing or storage, when wine is exposed to the undesirable activity of decarboxylase-positive microorganisms. Contamination may also occur from poorly sanitary conditions of both grapes and processing equipment. Although yeasts may also contribute to the final levels of biogenic amines in wines (Torrea and Ancín, 2002; Caruso et al., 2002), most biogenic amine contamination of wine is believed to take place during malolactic fermentation from the presence of lactic acid bacteria strains that produce enzymes which decarboxylate the corresponding precursor amino acids (Marcobal et al., 2006a). For example, in the production of the biogenic amine histamine, the enzyme involved is histidine decarboxylase, which acts on the amino acid precursor, histidine.

It is well known that among the species and strains of wine's lactic acid bacteria, some of them are practically unable to produce biogenic amines whereas others are

characterized for their high capacity of production. It is often to detect aminobiogenic strains among heterofermentative lactobacilli (*Lactobacillus hilgardii* y *L. brevis*) (Moreno-Arribas et al., 2000), although among histamine producers strains, some *Pediococcus* (Landete et al., 2005; Bodmer and Gafner, 2000) and *O. oeni* strains have been also isolated (Coton et al., 1998). The latter have also been found to be a putrescine producer (Marcobal et al., 2004; Marcobal et al., 2006b). Although it has been demonstrated that oenococci could produce histamine and putrescine, this property seems to be variable and is only present in some strains, which explains why in other studies, strains of biogenic amine-producing *O. oeni* have not been found (Constantini et al., 2006; Moreno-Arribas and Polo, 2008).

Molecular methods for the detection of biogenic amine-producing bacteria have been recently reviewed by Landete et al., (2007). PCR techniques have been developed to detect bacterial amino acid decarboxylases in a rapid, sensitive and accurate way. They cannot determine quantitative (or qualitative) amounts of biogenic amines, but can be used to estimate the potential risk for amine formation. *Multiple* PCR systems that use different pairs of primers to simultaneously detect lactic acid bacteria producers of histamine, tyramine and putrescine, the major wine biogenic amines have been developed (Marcobal et al., 2005b; Constantini et al., 2006). This system detects the presence of genes that encode the enzymes responsible for synthesizing these amines in bacteria. Quantitative PCR (or real time PCR) allows knowing the number of potential amine producers bacteria in wine, therefore it is an interesting tool to evaluate the risk for biogenic amine production from a specific bacteria population (Lucas et al., 2008).

From the wide range of techniques described in the literature to determine the concentration of biogenic amines in wines, reverse phase high-performance liquid chromatography (RP-HPLC) is one of most used in laboratories, since it is possible to

obtain valuable information about the presence and concentration of amines in musts and wines (Lehtonen, 1996; Torrea and Ancín, 2002; Marcobal et al., 2005a; Gómez-Alonso et al., 2007). Moreover, other chromatographic techniques applicable to biogenic amine analysis, such as micellar liquid chromatography (MLC) (Gil-Augustí et al., 2007) and liquid-chromatography-electrospray ionisation ion trap mass spectrometry (Millán et al., 2007) have been developed. Enzymatic methods (ELISA or other enzymatic tools) for histamine analysis able to determine levels of this amine in a short time and do not require sophisticated equipment have lately appeared (Marcobal et al., 2005). In recent years there has also been a growing interest in the use of capillary electrophoresis (CE) and different CE methods with diverse detection systems (Santos et al., 2004; Simó et al., 2008).

Regarding enological practices and technological factors that may influence biogenic amine formation in wines, special attention should be paid to some oenological practices frequently used to enhance wine complexity (flavour and aroma), but that also can increase the concentration of the precursor amino acids, such as the ageing of wines with lees or longer maceration times (Martín-Álvarez et al., 2006). Yeasts can indirectly play an important role on the production of biogenic amines by lactic acid bacteria, since they affect the amino acid composition during the alcoholic fermentation (Soufleros et al., 1998) or during autolysis (Moreno-Arribas et al., 1998; Villamiel et al., 2008). These amino acids can act as precursor for the formation of biogenic amines during malolactic fermentation and during wine ageing (Martín-Álvarez et al., 2006; Marques et al., 2008). Yeast and bacteria lees can also be a source for decarboxylase enzymes that could be involved in amine production (Marcobal et al., 2004).

The use of bacteria starter with specific characteristics such a very reduced or null capacity to produce biogenic amines, is the most popular strategy to control amine

production during winemaking. *O. oeni* commercial starters are selected on the basis of their oenological properties and among them, the absence of amino acid decarboxylase enzymes is an important requirement. Research performed in *in vitro* studies (Moreno-Arribas et al., 2003) and in industrial conditions during winemaking (Marcobal et al., 2006a) have shown the viability of using bacteria starters without the gene involved in biogenic amine production to prevent the formation of amines in wines. Moreover, it has been shown that the co-inoculation of *O. oeni* starters simultaneously to the alcoholic fermentation as opposite to the conventional inoculation once the alcoholic fermentation has been completed, is more effective to avoid the production of biogenic amines (Smit, 2007).

The use of different clarification substances and oenological adjuvants, such as bentonite PVPP at the normal dose used in winemaking can affect the concentration of biogenic amines in wines, since these compounds have the ability for the adsorption of some of them (Alcaide-Hidalgo et al., 2008). Also the type container employed during malolactic fermentation (stainless steel or oak barrel) seems to affect biogenic amine content of wines (Alcaide-Hidalgo et al., 2007a). Some alternative techniques for wine ageing based on the use of wood chips or oenological tannins, can also influence the amine production depending on the product type and on the dose of these compounds applied to the wines (García-Ruiz et al., 2008b).

Biogenic amine formation is also determined by wine parameters, such as pH, ethanol and SO_2, which have an important effect on the diversity of microorganisms, decarboxylase enzyme activity and decarboxylase gene expression (Lonvaud-Funel, 1999; Leitao et al., 2000; Mazzoli et al., 2008).

COMPOUNDS PRESENT IN WINE WITH POTENTIALLY BENEFICIAL EFFECTS ON HEALTH

Since the postulation of the French paradox in 1992 by Renaud and de Lorgeril (1992), moderate consumption of red wine has been associated with reduce risk of developing cardiovascular heart disease (CHD). The findings that constitute the French paradox for CHD were confirmed in the MONICA project, a worldwide study for cardiovascular diseases organized by the World Health Organization, that showed that the mortality rate from CHD was much lower in France, than in other industrialised countries such as USA and UK, despite the presence of high level of risk factors, such as cholesterol, diabetes, hypertension, and high intake of saturated fat. These effects were partly attributed to the high intake of wine and in general to the so-called Mediterranean-type diet, which is particularly abundant in fruits, vegetables and olive oil. These findings were already in agreement with a previous epidemiological study reporting an inverse association between moderate wine consumption and CHD (St leger et al., 1979). Later studies have also confirmed the results of Renaud et al. (1992). Gronbaek et al. (2000) found that all-cause mortality was reduced in wine but not in non-wine drinkers in a study including 24,523 subjects over 10 years. In a meta-analysis on 209,523 subjects, di Castelnuovo et al. (2002) also reported a larger benefit for wine (~22% reduction) than for beer. Wine drinkers were found to have a lower mortality risk than beer o liquor drinkers in a follow-up study including 128,934 subjects up to 20 years (Klatsky et al., 2003). The better effects of wine in comparison to other alcoholic beverages have been mainly attributed to the presence of antioxidant polyphenols that provide additional benefits. Other compounds such as low-molecular-weight peptides exhibiting antihypertensive activity could also contribute to these

beneficial effects. However, other prospective studies have indicated that the beneficial effects of alcohol intake were not dependent on the type of alcoholic beverage (Mukamal et al., 2003, Imhof et al., 2004). In a meta-analysis, moderate alcohol consumption (as much as 30 g alcohol/day) was related to lower risk (20-45% reduction) of CHD compared with abstention (Rimm et al., 1999). Discrepancies among studies have been attributed to the existence of many confounding variables related to life style and genetic factors. Health effects of moderate alcohol consumption (≤ 2 drinks/day) have been mainly related to an increase in HDL-cholesterol as well as to other protective mechanisms such as anti-platelet, anti-coagulatory, and anti-inflammatory effects (Rimm et al., 1999, Imhof et al., 2004). However, three or more drinks per day are associated with cardiomyopathy, hypertension, hypertriglyceridemia, and stroke. Although keeping in mind the importance of alcohol in the potentially beneficial effects of wine on human health, our dissertation will be focus on phenolic compounds and peptides as specific wine components with potentially beneficial effects.

Phenolic compounds

The term "phenolic" or "polyphenolic" describes the compounds that possess a benzenic ring substituted by one or several hydroxyl groups (-OH). Based on their carbon skeleton, polyphenols are classified in non-flavonoid and flavonoid compounds. Main non-flavonoid compounds present in wine are phenolic acids (hydroxybenzoic and hydroxycinnamic acids), as well as others phenolic derivatives such as stilbenes **(Figure 3)**. Flavonoid compounds present in wine mainly include the flavonols, flavan-3-ols and anthocyanins, as well as flavanonols and flavones, in lower proportion

(Figure 3). Due to their chemical structure, containing several hydroxyl groups, stilbenes and flavonoids have been demonstrated to be the most bioactive compounds in wine.

Hydroxylated stilbenes, as previously decribed in section #, are phytoalexins synthesized by the plant, especially in the skins, leaves and roots, in response to fungal infections and ultraviolet (UV) light (Jeandet et al., 1991; Korhammer et al., 1995). Stilbenes present in wine include *trans* and *cis* resveratrol (3,5,4'-trihydroxystilbene), as well as their glucose derivatives (*trans* and *cis* piceid). A series of mono-, di-, and tetrahydroxystilbenes have been also identified in wine (Lamikanra et al. 1996; Ribeiro de Lima et al. 1999; Baderschneider and Winterhalter, 2000). Considering that grapes and their derived products are the most important dietary sources of stilbenes (Mattivi et al., 1995), resveratrol metabolites have been recently proposed as a biomarkers of moderate wine consumption in humans (Zamora-Ros et al., 2006).

In relation to the flavonoid phenolic compounds found in wine, flavonols are yellow pigments mainly located in the vacuoles of the epidermal tissues. In wine they exist as the 3-*O*-glycosides of four main aglycones: myricetin, quercetin, kaempherol and isorhamnetin **(Figure 3)**. Flavan-3-ols or flavanols are found in wine in monomeric, oligomeric or polymeric forms in the solid parts of the grape; the latter two forms are also known as proanthocyanidins or condensed tannins. Flavanol monomeric units include (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-*O*-gallate, (+)-gallocatechin and (-)-epigallocatechin **(Figure 3)**. Proanthocyanidins are constituted of flavanols units linked by C4-C6 or C4-C8 bonds. Proanthocyanidins with a mean degree of polymerization between 3 and 7 units have been reported in red wine (Fulcrand et al., 1999). Anthocyanins are pigment located in the grape skins of red grape varieties. Main anthocyanins identified in wine include the 3-*O*-monoglucosides and the 3-*O*-acylated

monoglucosides of five main anthocyanidins: delphinidin, cyanidin, petunidin, peonidin and malvidin **(Figure 3).** These compounds could be acylated in the C-6 position of the glucose molecule by esterification with acetic, *p*-coumaric and caffeic acids. Both, anthocyanins and flavanols are the most abundant flavonoids in red wines and they are largely responsible for the color, bitterness, astringency and chemical stability of red wines. A glass of red wine provides approximately 100 mg of polyphenols. In comparison to red wines, white wines are devoid of anthocyanins, and due to absence of skins and seed contact during maceration and fermentation in white wine technology, they possess a lower content of phenolic compounds, in particular of flavonoids.

In vitro and animal model studies have demonstrated that red wine polyphenols are protective agents against the development and progression of atherosclerosis (Dell'Agli et al., 2004). This protection is related to: *i) inhibition of LDL oxidation* (Frankel et al., 1993; Lourenço et al., 2008); *ii) vasorelaxation* (reduction of blood pressure; endothelium-dependent vasorelaxation by increased generation of NO, endothelium-derived hyperpolarizing factor and prostacyclin, or by reduction of the vasoconstrictor endothelin-1) (Fitzpatrick et al., 2000; Diebolt et al., 2001; Ndiaye et al., 2003, Schramm et al., 1997; Corder et al., 2001); *iii) inhibition of vascular smooth muscle cell migration and proliferation* [by possible inhibiting platelet-derived growth factor (PDGF) β-receptor and vascular endothelial growth factor (VEGF)] (Rosenkranz et al., 2002; Ferrera et al., 1999); and *iv) inhibition of platelet aggregation* (by stimulation of the synthesis of prostacyclin; suppression of platelet activation response to epinephrine; increase of platelet c-AMP levels) (Gryglewski et al., 1987; Rein et al., 2000; Russo et al., 2001). Administration of red wine, dealcoholized wine or grape juice has also been shown to attenuate the development of atherosclerotic lessions (da Luz., 1999; Vinson et al., 2001) and to prevent thrombosis (Demrow et al., 1995) in

experimental animals. Red wine polyphenols also exhibit antiviral (Takechi et al., 1985), antibacterial (Cueva et al., 2008), anticancer (Franke et al., 2002; Weyant et al., 2001) and antiulcer (Saito et al., 1998) activities. Finally, the implication of red wine polyphenols in the modulation of gene expression has also been reported (Sen and Bagchi, 2001).

The health effects observed in *in vitro* and animal model studies have been partially confirmed in human subjects. A summary of short-term human intervention studies related to wine polyphenols is presented in **Table 2**. These studies have focused on confirming the hypothesis that: *i)* red wine provides more beneficial effects than other alcoholic beverage (red wine vs. spirits or beer); *ii)* red wine properties are beyond alcohol (red wine vs dealcoholized wine or grape juice); *iii)* red wine leads to greater beneficial effects than white wine, and *iv)* polyphenols are responsible for red wine properties. Most studies have been conducted on healthy men only, or on healthy men and women, with the exception of one study that was performed on healthy women (Sacanella et al., 2007). Intervention periods ranged between 14 and 30 days, although some acute intake (1 day) studies have also been performed. Alcohol doses were on the order of 20-40 g ethanol/day. Biomarkers significantly affected in these trials are related to red wine: *i) antioxidant effect* (inhibition of the LDL oxidation; reduction of plasma lipid peroxides, thiobarbituric acid-reactive substances, conjugated dienes, and urine isoprotanes levels; increase in plasma antioxidant capacity, plasma polyphenol content and LDL polyphenols); *ii) effects on lipid metabolism* (increment in plasma HDL cholesterol; reduction of LDL/HDL ratio and LDLox antibody); *iii) anti-thrombotic effects* (reduction of trombine-induced and collagen-induced platelet aggregation; reduction of plasmatic levels of tromboxane B2, fribinogen, factor VII, t-

PA, PAI); i*v) effects on vascular function* (reduction in flow-mediated dilatation of the brachial artery), and *vi) anti-inflammatory effects* (reduction of the expression of adhesion molecules on monocyte surface, including LFA-1, Mac-1, VLA-4 andMCP-1; reduction of serum levels of hs-CRP, VCAM-1, ICAM-1 and E-selectin; increment of TGFβ1). The anti-inflammatory effects of white sparkling wine (cava) in comparison to gin have also been determined and were on favour of cava (Vázquez-Agell et al., 2007). However, it is important to highlight that the other studies failed to find positive outcomes for some parameters after red wine consumption. According to Djoussé et al. (1999), no significant changes were found in flow-mediated dilation of the brachial artery when red wine was drunk together with a fatty meal. Watzl et al. (2002, 2004) did not find changes in anti-inflammatory biomarkers after acute and chronic consumption of red wine. Van deer Gaag et al. (2000a,b) found an increase in plasma homocysteine levels after red wine consumption and no significant changes in the activity of antioxidant enzymes or plasmatic levels of some antioxidant vitamins.

The *in vivo* physiological importance derived from wine consumption is dependent on the bioavailability (absorption, distribution, metabolism and excretion) of wine polyphenols. The metabolic pathway of polyphenols is well established (Scalbert and Williamson, 2000). The bioavailability of wine polyphenols is influenced by their chemical structure. Monomers and dimeric procyanidins in a lower extent, are directly absorbed in the small intestine, whereas oligomers with a mean degree of polimerization (mDP)>3 and polymeric flavanols (proanthocyanidins) are not absorbed in their native forms or degraded under the acidic conditions of the stomach *in vivo* (Rios et al., 2002). These compounds reach the colon where they are metabolized by the intestinal microbiota into various phenolic acids, including phenylpropionic, phenylacetic and benzoic acid derivatives that can be further absorbed by enterohepatic recirculation via

the bile duct (Manach et al., 2004). In the other hand, glycosilated polyphenols such as resveratrol glucosides and anthocyanins, must be first hydrolyzed by intestinal β-glucosidases before they can be absorbed (Manach et al., 2004). However, in the case of wine anthocyanins, it has been demonstrated that they can be absorbed in their glycosilated form after oral ingestion (Matsumoto et al., 2001; Cao et al., 2001; McGhie et al., 2003).

After passing across the small intestine brush border, polyphenols are extensively conjugated first in the small intestine and then in the liver into methyl, glucoronide and sulphate derivatives by the enzymes catechol-*O*-methyltransferase, UDP-glucuronosyl transferase, and phenol sulfotransferases, respectively (Scalbert and Williamson, 2000). In general, glucuronide metabolites are the most abundant metabolites in plasma and only trace amounts of native forms are found. However, in the case of anthocyanins, although the existence of methyl, glucuronide and sulfate derivatives has been demonstrated (Wu et al., 2002; Felgines et al. 2003), intact glycosides were the only metabolites reported in previous studies, probably due to their degradation during sample conservation (Manach et al., 2004). This fact together with other issues related to the use of inappropriate analytical methods may have resulted in an underestimation of anthocyanin bioavailability in early studies.

Pharmacokinetic studies indicate that after the ingestion of 35 mg of catechin supplied in the form of red wine (120 mL), the maximum concentration (C_{max}) of catechin in plasma was on the order of 0.077-0.091 μmol/L, and was achieved at a mean time (T_{max}) between 1.44-1.50 h. Urinary excretion ranged between 3-10% of the intake and the elimination half-life was approximately 3 h (Donovan et al., 1999, 2002; Bell et al., 2000). Concerning proanthocyanins, up to date there is no literature data of the absorption of these compounds after wine consumption. A plasma concentration of

0.0011 μmol/L of procyanidin B1 has been reported after the ingestion of grape seed extract (18 mg of procyanidin B1) in humans (Sano et al., 2003). In relation to the microbial metabolism of proanthocyanins into phenolic acids, Gonthier et al. (2003) have shown that the DP has an inverse association with the extent of microbial degradation in rats fed purified monomeric, dimeric, trimeric or polymeric flavanols. In comparison with flavanols, anthocyanins are more rapidly absorbed. After the ingestion of 68 mg of malvidin-3-glucosides in the form of red wine (500 mL), T_{max} in plasma and urine was attained at 0.83h and <3h, respectively (Bub et al., 2001); data which seem to be consistent with absorption in the stomach. However the C_{max} was much lower (1.4 nmol/L) than for flavanols, as well as the urinary excretion (0.016%) (Bub et al., 2001). In another study, higher urinary excretion were found (1.5-5.1 %) after the consumption of 300 mL red wine (218 mg of total anthocyanins) (Lapidot et al., 1998). The bioavailability of resveratrol has not been determined after the consumption of red wine in humans. Oral administration of resveratrol (0.026 mg total resveratrol) to rats in the form of wine (4 mL), gave resveratrol levels of 0.1 μmol/L, which is similar to that of catechin (Bertelli et al., 1996). A larger number of studies should be carried out in order to acquire more information about the metabolism of red wine polyphenols after acute or chronic consumption of wine and its correlatation with the observed *in vivo* effects.

Peptides

Peptides are important wine compounds involved in different properties, such as tensioactive (González-Llano et al., 2004), sensorial (Desportes et al., 2001) or functional (Pozo-Bayón et al., 2007) properties. In addition, they can act as yeasts and bacteria nutrients (Feuillat et al., 1977; Alexandre et al., 2001; Manca de Nadra et al., 2005;

Remize et al., 2006). However, wine peptides are the least known nitrogen compounds. The main reason for the dearth of studies on this fraction is due to their complexity and lack of specificity of the techniques used for their analysis.

Due to the wide range of structures (amino acid composition and sequence) in which these compounds may appear, peptides form a heterogeneous group of compounds. Generally speaking, the term oligopeptides, or low-molecular-weight peptides, refers to peptides with 10 or fewer residues of amino acids, while the term polypeptides is used for peptides with more amino acids. Although the transition point from polypeptide to protein is not well defined, proteins are normally considered to have at least 100 residues of amino acids (Mr> 10,000).

In recent years, several studies have focused on their origin and identification (Acedo et al. 1994; Moreno-Arribas et al. 1998) and in some cases, the sequence of amino acids have also been elucidated (Yahai et al. 2003; Person et al. 2004; Desportes et al. 2001). Some of these studies have shown that the amino acids aspartic acid and/or asparagine and glutamic acid and/or glutamine are involved in the majority of wine peptides, independently of the type of wine or the analytical methodology employed in the analysis.

The wine peptide fraction is continuously changing both qualitatively and quantitatively. Some wine peptides come from the must, but most of them appear in the different stages of wine production. During alcoholic fermentation, especially if it takes place in nitrogen-deficient media, there is a reduction in their concentration. In the final stages of fermentation, peptides are released into the medium, reaching the maximum concentration after cell death (Usseglio-Tomasset and Bossia, 1990). In fact, it has been shown that, at least part of the peptide fraction of wines is derived from the yeasts involved in the alcoholic fermentation. These peptides can be hydrolysed by the action

of extracellular enzymes, being transformed into low molecular weight peptides (Moreno-Arribas et al., 1996; Martínez-Rodríguez et al., 2000, 2001). The existence of endo and exocellular proteases has been evidenced under winemaking conditions (Feuillat et al., 1980; Alexandre et al., 2001). This process is even more important in winemaking technologies based on ageing the wines with yeasts for long periods of time, such as biologically aged wines (Dos Santos et al., 2000; Villamiel et al., 2008) and sparkling wines manufactured by the *traditional* method (Moreno-Arribas et al., 1996, 1998 a,b).

Also, some wine lactic acid bacteria strains have shown proteolytic activity under winemaking conditions (Feuillat et al., 1980; Manca de Nadra et al., 1997; Leitào et al., 2000). This activity has been found even in the presence of ethanol and SO_2 (Manca de Nadra et al., 2005) and has been reported to be greater in red than in white wines (Manca de Nadra et al., 1999). The proteolytic activity is a very important characteristic for some bacteria strains allowing them to growth in nitrogen-deficient media, and to carry out the malolactic fermentation. According to Remize et al. (2005) and Remize et al., (2006), peptides from 0.5 to 10 kDa seem to be more favourable for the growth of wine lactic acid bacteria than other nitrogen sources (<0.5 kDa). Alcaide-Hidalgo et al., (2008) have observed a reduction in peptides during the malolactic fermentation of red wines followed by an increase during the ageing in barrels, that was more pronounce when the aging was in presence of lees. The occurrence of proteolytic activity in all the stages of wine manufacture may be responsible for the wide range of peptides that can be present in a wine at any time, and the discrepancies in most of the studies related to their identification.

Low molecular weight peptides can be implicated in important biological activities such antimicrobial and antioxidant. The antihypertensive activity is the

bioactive property of peptides that has been most studied . The increase in hypertension rates of the population in recent years, which is considered as one of the commonest chronic diseases in developed countries, has favoured these types of studies. Most of the antihypertensive peptides derived from foods act by inhibiting angiotensin-converting enzyme (ACE). In the organism, ACE is present in a large number of organs and tissues, such as lung, intestine, kidneys, testicles, heart, skeletal muscle, pancreas, spleen or placenta. It is thought that the ACE located in the lung belongs to the renin-angiotensin system, one of the main mechanisms that regulates blood pressure in the body. The mechanism of action of the enzyme operates at two levels, on one hand, it catalyses the transformation of angiotensin I to angiotensin II, which has a strong vasoconstrictor action in the sympathic nervous system and on the blood vessels. One the other hand, ACE inactivates bradykinin, a strong vasodilator and natriuretic. Both mechanisms cause an overall increase in blood pressure. The use of ACE inhibitors as active ingredients is the strategy of current antihypertensive drugs (captropil), to control blood pressure and other related diseases.

It has been shown that some peptides from fermented foods products can act by inhibiting the activity of the ACE enzyme, and, therefore, causing a lowering of blood pressure after intake (Hartmanna and Meisela, 2007). However, afterwards these peptides must be resistant to the action of gastric enzymes and must be absorbed to have an effect. Therefore, after isolating and identifying peptides with *in vitro* biological activity, *in vivo* studies are required to establish their true efficacy.

In wines, the first study focused on the occurrence of antihypertensive peptides was carried out by Takayanagi and Yokotsuka (1999), who determined the inhibitory activity of ACE (IACE) in two red wines and four white wines and in grapes of the red

Muscat Bailey A variety and during fermentation of the must. They showed a greater ACE inhibitor activity in red wines than white wines and a decrease in IACE during fermentation, although not providing any clear explanation for that.

In long-term trials carried out on normotensive and spontaneously hypertensive rats, Perrot et al., (2003) showed that the extract from the low molecular weight fraction of a Champagne wine had antihypertensive activity in hypertensive rats but no effects in normotensive rats. However, owing to the complexity of this fraction, the authors indicated that this finding could not be only attributed to a single compound present in this fraction. Pozo-Bayón et al., (2005), showed that amino acids aspartic acid and/or asparagine and glutamic acid and/or glutamine and valine, formed part of 5 from the 6 fractions from white and red wines with IACE activity, while threonine and alanine formed part of 4 of them.

Due to the abundance of phenols and peptides in wines, it is difficult to attribute the IACE activity to one of these groups since both of them include individual compounds with this activity (Ling, 2003; Zhang et al., 2003). Although Pozo-Bayón et al., (2005) demonstrated that red wine fractions with high peptide and relative low phenolic contents highly contributed to IACE activity, it has also been found that red wines have greater ACE inhibitory activity than white wines (Takayanagi and Yokotsuka, 1999), suggesting the contribution of phenolic compounds to this activity. Lin (2003) has shown that phenols can be conjugated with proteins and produce non-competitive inhibition of the ACE. It is, therefore, necessary to carry out more studies to identify the compounds responsible for this activity in wines. Recently, ACE inhibitory activity has also been found in peptides released from *Saccharomyces cerevisiae* in a model wine (Alcaide-Hidalgo et al., 2007b). In this assay, there were not phenolic compounds, thus the IACE activity was exclusively attributed to yeast peptides.

Moreover, the same rich peptides fractions also showed antioxidant activity, suggesting that peptides released by *S. cerevisiae* during autolysis under wine conditions could present multifunctional activities.

Recently, prolylendopeptidase inhibitory peptides (PEP) have also been found in wines (Yanai et al., 2003). PEP may have a role in the degradation of biologically-active peptides containing proline, such as oxytocin, vasopressin, P substance, bradykinin, neurotensins and angiotensins. Two inhibitory peptides have been isolated and characterized, Pep A (Val-Glu-Ile-Pro-Glu) and Pep B (Tyr, Pro, Ile, Pro, Phe). Both of them showed PEP inhibition thereby suppressing the degradation of neuropeptides, vasopressin, substance P and the fragments 8-13 of neurotensin, which are involved in memory and neural communication.

CONCLUSIONS AND FUTURE OUTLOOK

The aim of the present review has been to gather, from an integrated perspective, the scientific information concerning wine health and safety, a matter of emergent interest for researchers and consumers.

There is a wide variety of compounds that can affect wine safety. However to solve these problems there are different strategies currently available. For example, it should be pointed out that a large part of today's advance in control and prevention strategies for wine contaminants has been possible thanks to the funding from several research frameworks (special EU research projects and other bilateral grants). Specific funds to increase the scientific knowledge related to the presence of OTA, biogenic amines and sulphur dioxide in wines, among others, have concluded in relevant

scientific outputs. As a consequence, the winemaking industry has specific knowledge currently available to control and/or prevent the formation of harmful compounds.

Great progress has also been done in recent years, trying to elucidate the compounds associated to the health benefits of moderate wine consumption and their action mode. Most of these advances have been focused on the study of wine phenolic compounds, confirming their key role in some healthy aspects derived from wine consumption.

Laboratory, but mainly clinical research, will continue exploring the efficacy of these important wine constituents in the human body, their bioavailability and action mechanisms. In the future, some promising applications of wine phenolics, such as their use in prevention or conventional therapies to fight cancer and infections will gain interest. Clinical studies of how the human body interact with wine constituents and how such interactions can be modulated, will continue. Moreover, there is growing evidence of the biological properties of other wine constituents, such as peptides. Nevertheless, due to their complexity and low abundance in wines in comparison with other fermented foods, the scientific information regarding peptides as bioactive agents in wines is still scarce. Therefore, it is foreseeable that in the next years some progress related to the identity and health functions of specific wine peptides would increase.

As a summary, it is possible to confirm for consumers, that utmost efforts are being made in research, technology and administrative control to ensure that wine is a safe, pleasant and healthy drink when consumed responsibly.

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Table 1. Summary of the most important measures to prevent or reduce OTA accumulation in wines, according the *Code of Sound Vitivinicultural Practices* of the OIV (OIV, 2005, Resolution VITI-OENO 1/2005)

Cultivation practices in the vineyards

- To train producers with regards to risk of mould and mycotoxins, the identification of ochratoxigenic fungus and period of infection, and knowledge of preventive measures to be applied to vineyard and wineries

Practices at harvest

- To train the harvesting staff to reject rotten bunches, particularly those affected by dark brown black moulds
- To vinify the mechanical harvested grapes separately, when the sanitary quality of the crop is poor

Treatment at the winery

- To monitor, in large wineries and cooperatives, the sanitary quality of the grapes in order to favour with fair prIcing the sanitary quality of grapes, and to process the grapes accordingly
- To avoid long periods of maceration and to use enological adsorbents, such as activated charcoal or yeast hulls, in red wine, and bentonite, in white wine, when the crop has a relevant percentage of rotten grapes
- To perform a rapid grape drying and avoid water condensation overnight for grapes used in the vinification of dessert wines
- To implement a complete HACCP plan, from the vine to the bottled wine or raisin, in the wine regions where the OTA occurrence in higher

Table 2. Human intervention studies related to wine consumption.

Reference	Subjects*	Intervention test products	Dose/day	Duration (days)	Positive outcomes	Negative outcomes
Lavy et al. (1994)	20 men	-Red wine -White wine	400 mL (40g ethanol/day)	15	-Red wine: Increment in plasma HDLc and apo A1	No changes in plasma total cholesterol, LDLc, VLDLc
Pace-Asciak et al. (1996)	24 men	-Red wine -White wine -Grape juice -Grape juice enriched with resveratrol	400 mL (40g ethanol/day)	30	-Red wine/white wine: reduction of trombine-induced platelet aggregation and plasmatic levels of tromboxane B2 -White wine: reduction of ADP-induced platelet aggregation -Enriched grape juice: Reduction of trombine-induced platelet aggregation	-Red wine: no reduction of ADP-induced platelet aggregation
Pellegrini et al. (1996)	11 men	-Red wine -Fruit juice + alcohol -Dealcoholized red wine	-320 mL (30g ethanol/day) -320 mL (30g ethanol/day) -320 mL	30	-Red wine / fruit juice+alcohol: Reduction in collagen-induced platelet aggregation and fribinogen levels	No changes in ADP-induced platelet aggregation, t-PA antigen, vWF and plasminogen levels
Miyagi et al. (1997)	20 men	-Red wine -White wine -Beer -Grape juice	-300 mL -450 mL -750 mL -300 mL	1	-Red wine: Inhibition of the LDL oxidation after 1 and 2 h of ingestion	
Nigdikar et al. (1998)	9 men	-Red wine -White wine -Red wine polyphenols (capsule) -Red wine polyphenols in white wine -Alcoholic drink	-375 mL -375 mL -1g/day= 375 ml red wine -1g/day -40g ethanol /day	14	-Red wine, red wine polyphenols, red wine polyphenols+white wine: Reduction of plasma lipid peroxides, thiobarbituric acid-reactive substances, and conjugated dienes. Increase in plasma and LDL polyphenols -White wine: Increase in thiobarbituric acid-reactive substances	No changes in plasma triacylglycerol, LDL or HDL cholesterol
Serafíni et al. (1998)	10 men	-Dealcoholized red wine - Dealcoholized white wine -Water	113 mL	1	-Dealcoholized red wine: Increment in plasma antioxidant capacity and polyphenol content after 50 min of ingestion	

Reference	Subjects*	Intervention test products	Dose/day	Duration (days)	Positive outcomes	Negative outcomes
Ursini et al. (1998)	9 men	-fatty meal -red wine+ fatty meal	300 mL	1	Red wine+fatty meal: Reduction of post-prandial plasma lipid hydroperoxide levels	
Djoussé et al. (1999)	13 men/women	-Red wine+fatty meal (0.8 g fat/kg body weight) -Isocaloric beverage	3mL/kg	1		No significant changes in FMD of the brachial artery after consumption of red wine +fatty meal
Stein et al. (1999)	15 men/women with coronary artery disease	Grape juice	7.7±1.2 mL/kg	15	Improvement of FMD and decrease of LDL susceptibility to oxidation	
Cuevas et al. (2000)	6 men	-Red wine + fatty meal -Fatty meal	240 mL	30	-Red wine: reduction in FMD of the brachial artery	
van deer Gaag et al. (2000a)	9 men	-Red wine -spirits -Beer -Water	40 g ethanol /day	21	-Beer: Decrease in plasma homocysteine levels	-Red wine/spirits: Increment in plasma homocysteine levels
van deer Gaag et al. (2000b)	11 men	-Red wine -spirits -Beer -Water	40 g ethanol /day	21		No effets on the enzyme activities of serum glutathion peroxidase, erythrocyte glutathion reductase and superoxide dismutase, and plasma concentrations of α-and γ-tocopherol, lutein, zeaxantin, β-cryptoxanthin, lycopene and α-carotene
Pignatelli et al. (2002)	20 men/women	-Red wine -White wine	300 mL	15	-Red wine: higher reduction of collagen-induced platelet aggregation	
Watzl et al. (2002)	6 men	-Red wine -12% ethanol dilution	500 mL	1		No effects on: TNF-α, IL-2, IL-4, phagocity activity of neutrophilis and lymphocyte proliferation
Watzl et al. (2004)	24 men	-Red wine -12% ethanol dilution -Dealcoholized red wine -Red grape juice	500 mL	15		No effects on: TNF-α, TNF-α mRNA, IL-2, IL-4, TGF-β, phagocity activity of neutrophilis, lymphocyte proliferation, lytic activity of natural killer cells, and percentage of apoptotic lymphocytes

Reference	Subjects*	Intervention test products	Dose/day	Duration (days)	Positive outcomes	Negative outcomes
Badía et al. (2004)	8 men	-Red wine -Gin	-320 mL -100 mL (30 g ethanol/day)	28	-Red wine: Reduction of the expression of VLA-4 in monocyte surface. -Both: Reduction of the TNF-α induced adhesion of monocyte to an endothelial cell line. More pronounce for red wine.	
Estruch et al. (2004)	40 men	-Red wine -Gin	-320 mL -100 mL (30 g tanol/day)	28	-Red wine: reduction in the expression of LFA-1, Mac-1, VLA-4 and MCP-1 on monocyte surface. Reduction of serum levels of hs-CRP, VCAM-1 and ICAM-1. -Both: Reduction in C-reactive protein and IL1-α levels	
Papamichael et al. (2004)	16 men/women	-Cigarette (1) -Cigarette (1) +red wine -Cigarette (1) + dealcholized red wine	250 mL	1	Cigarette smoking + red wine or dealcholized red wine: No changes in flow mediated dilatation after 15, 30, and 60 minutes after the inhalation of smoke compared to baseline levels	
Avellone et al. (2006)	48 men/women	-Red wine	250 mL	30	Reduction of LDL/HDL ratio, fibrinogen, factor VII, C-reactive protein, LDLox antibody. Increment in HDLc, apoA1, TGFβ1, t-PA, PAI and plasma antioxidant capacity	
Pignatelli et al. (2006)	20 men/women	-Red wine -Alcohol wine	300 mL	15	-Both: Increment in plasma antioxidant capacity and decrease in urine isoprotanes levels.	
Sacanella et al. (2007)	35 women	-Red wine -White wine	20g ethanol/day	30	-Red wine: Reduction of serum levels of VCAM-1 and E-selectin. -Both: Increment in HDLc. Reduction of serum concentrations of hs-CRP, ICAM-1, CD40L, and IL-6	

Reference	Subjects*	Intervention test products	Dose/day	Duration (days)	Positive outcomes	Negative outcomes
Vázquez-Agell et al. (2007)	20 men	-Sparkling white wine (cava) -Gin	-300 mL -100 mL (30g ethanol/day	28	-Cava: Reduction of the expression LFA-1, VLA-4, SLe^x and CD40 on monocyte surface. Reduction of serum levels of hsCRP, ICAM-1, IL-6, MCP-1 and CD40L -Gin: Reduction of while only SLe^x on monocyte surface. -Both: reduction of serum levels of VCAM-1, E-selectin and P-selectin.	
Imhof et al. (2008)	49 men/women	Red wine (12.5% alcohol; 275 mg/L polyphenols) -Ethanol (concentration 12.5% alcohol) -Beer (5.6% alcohol; 160 mg/L polyphenols) -De-alcoholized red wine (275 mg/L polyphenols) -De-alcoholized beer (171 mg/L polyphenols) -Water	30g ethanol/day for men and 20 g/d for women	21	-Ethanol/ de-alcoholised red: Reduction on MCP-1-induced monocyte migration and in FMLP-induced migration	No effects on MCP-1 receptor expression
Spaak et al. (2008)	30 men/women	-Red wine -Ethanol -Water	-120 ml -44 ml (one drink)	1	-Ethanol/wine: Increase in cardiac output and MSNA after 2 drinks of each. Increase in brachial artery diameter after both one and two drinks. -Red wine: Increment in heart rate and attenuation in FMD after 2 drinks.	No effects on blood pressure

*When not stated, studies were conducted on healthy subjects

ADP: adosin diphosphate; ApoA1: apoliprotein A1; FMD: flow mediated vasodilation; FMLP: N-formyl-methionyl-leucyl-phenylalanine; HDLc: high density lipoprotein cholesterol; hs-CRP: High-sensitivity C-reactive protein; ICAM-1: intercellular adhesion molecule-1; IL-2, IL1-α, IL-4, IL-6: interlukin 2, 1α, 4, 6; LDLc: low density lipoprotein cholesterol; LDLox: Oxidized LDL; LFA-1: lymphocyte function-associated antigen-1; Mac-1: CD11b/CD18; MCP-1: monocyte chemoattractant protein; MSNA: muscle sympathetic nerve activity; PAI : plasminogen activator inhibitor; SLe^x: Sialyl-Lewis^x; TGFβ1: transforming growth factor β1; TNF-α: tumor necrosis factor α; t-PA: tissue-type plasminogen activator antigen; VCAM-1: vascular adhesion molecule-1; VLA-4: very late activation antigen-4; VLDLc: very low density lipoprotein cholesterol; vWF: von Willebrand factor

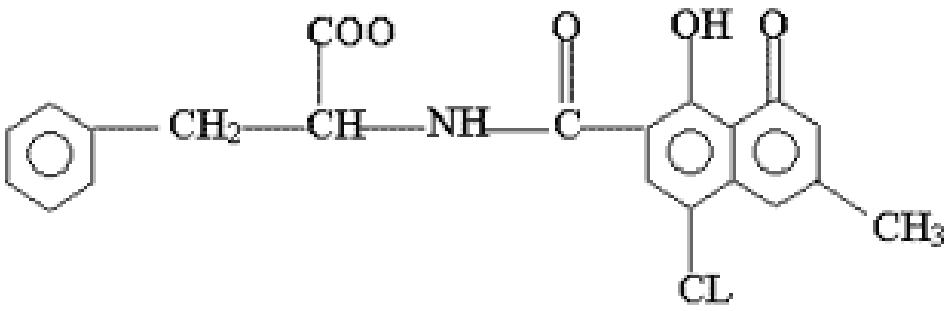
FIGURE LEGENDS

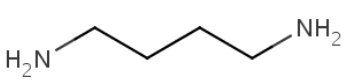
Figure 1. Chemical structure of ochratoxin A.

Figure 2. Chemical structure of main biogenic amines present in wine.

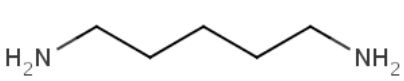
Figure 3. Chemical structure of main non-flavanoid and flavanoid phenolic compounds present in wine.

Figure 1

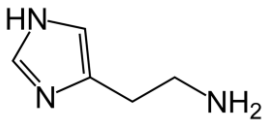




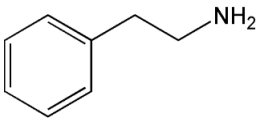
Putrescine



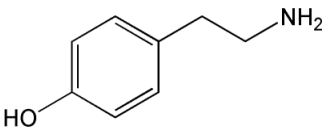
Cadaverine



Histamine



Phenylethylamin



Tyramine

1

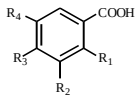
Figure 2

2

Figure 3

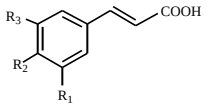
NON-FLAVANOID PHENOLIC COMPOUNDS

Hydroxybenzoic acids



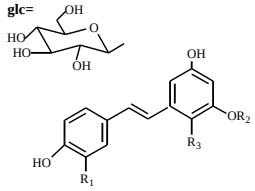
Benzoic acid	R_1	R_2	R_3	R_4
p-Hydroxybenzoic	H	H	OH	H
Protocatechuic	H	OH	OH	H
Vanillic	H	OCH_3	OH	H
Gallic	H	OH	OH	OH
Syringic	H	OCH_3	OH	OCH_3
Salicilic	OH	H	H	H
Gentisic	OH	H	H	OH

Hydroxycinnamic acids



Hydroxycinnamic acid	R_1	R_2	R_3
p-Coumaric	H	OH	H
Caffeic	OH	OH	H
Ferulic	OCH_3	OH	H
Sinapic	OCH_3	OH	OCH_3

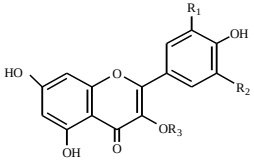
Stilbenes



Stilbene	R_1	R_2	R_3
Trans-resveratrol	H	H	H
Trans-resveratrol-3-*O*-glucoside (piceid)	H	glc	H
Trans-resveratrol-2-*C*-glucoside	H	H	glc
Trans-astringin	OH	glc	H

FLAVANOID PHENOLIC COMPOUNDS

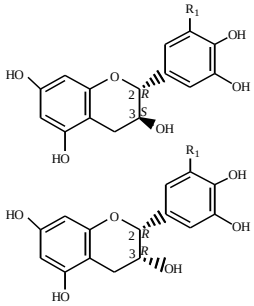
Flavonols



Flavonol	R_1	R_2	R_3
Kaempferol	H	H	H
Kaempferol-3-*O*-glucoside	H	H	glc
Kaempferol-3-*O*-galactoside	H	H	gal
Kaempferol-3-*O*-glucuronide	H	H	gluc
Quercetin	OH	H	H
Quercetin-3-*O*-glucoside	OH	H	glc
Quercetin-3-*O*-glucuronide	OH	H	gluc
Myricetin	OH	OH	H
Myricetin-3-*O*-glucoside	OH	OH	glc
Myricetin-3-*O*-glucuronide	OH	OH	gluc
Isorhamnetin	OCH_3	H	H
Isorhamnetin-3-*O*-glucoside	OCH_3	H	glc

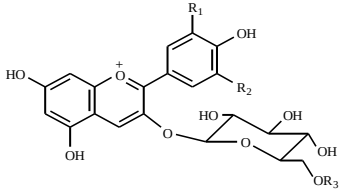
gal= galactose; glc= glucose; gluc= glucuronide acid

Flavan-3-ols or flavanols



Flavan-3-ol	R_1	C-2	C-3
(+)-Catechin	H	*R*	*S*
(+)-Gallocatechin	OH	*R*	*S*
(-)-Epicatechin	H	*R*	*R*
(-)-Epigallocatechin	OH	*R*	*R*

Anthocyanins



Anthocyanidin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

R_3= -CO-CH$_3$ **(-acetyl)** -CO-CH=CH-  **(-*p*-coumaroyl)** -CO-CH=CH-  **(-caffeoyl)**