Biochemistry of wine

- Statistical-epidemiological investigations have demonstrated a reduced incidence of cardiovascular diseases and related complications in certain regions of southern France, in spite of the high atherogenic fatty acids (saturated) consumption. This was in contrast with the high impact of the same diseases in other European and American populations, who consumed equivalent amounts of the same fats.

- This difference was immediately attributed to the usual consumption of wine of French people, as a protective factor against atherosclerosis and its related cardiovascular damages.

Wine as a food

- Although it is considered one of the typical agro-food products of the area, wine is gradually losing its food connotation, while it is taking cultural, hedonistic and social values.

- Nowadays wine seems to be considered the symbol of a cultural recovery of traditions and region values, in clear contrast with the trend of the standardization of tastes and values, driven by globalization, consumerism and mass communication.

- Epidemiological studies have strongly stimulated an already established line of scientific research that underlie the relationship between wine and health.

- To understand the characteristics of the wine as food and the latest scientific hypotheses about its antioxidant properties, it is necessary to present the knowledge acquired in the biochemistry of food.

The Mediterranean diet

- The term Mediterranean diet was derived from the eating habits of some Mediterranean populations (southern Italy and islands, Greece and islands), which in the early 50's showed a very low incidence of cardiovascular diseases, with respect to the United States of America.

- At that time, the Mediterranean populations used to consume a large variety of vegetables, fruits, grains and legumes, and fish; they cooked food using olive oil; they ate little meat, only during holidays, they used some cheese to flavor some dishes, and a few eggs to prepare omelets and herb tarts. The Mediterranean diet included one or two glasses of wine during meals.

- As you can see, the French (or Mediterranean) Paradox was anticipated by the studies on the Mediterranean diet, which had closely linked the wine, an old Mediterranean drink, with some equally old, traditional Mediterranean food: cereals, fresh vegetables, oil and fish.

The Mediterranean diet

MEAT
confectionery

Few times a month

Eggs
Poultry
Fish

Few times a week

Fruits, legumes,
vegetables,
Dried fruits

Everyday

Cereals, Pasta, potatoes and bread

Main constituents of wine

Constituents	Amount (g / l)
Water	750-900
Ethyl Alcohol	70-130
Methyl Alcohol	0,02-0,2
Higher Alcohols	0,1-0,5
Glycerol	4-15
Sugars (Glucose e Fructose)	Traces in dry wines Variable amounts in sweet wines
Tartaric acid	2-5
Malic Acid	0-7
Citric Acid	0,1-0,5
Succinic Acid	0,5-1,5
Lactic Acid	1-5
Acetic Acid	0,2-0,9
Phenolic compounds (tannins, etc.).	0,2-3
Nitrogen Compounds	0,05-0,9
Mineralis(ash?)	2-3

Ethanol as energetic nutrient

- Alcohols and, more importantly, the products of their oxidation –the aldehydes-, are toxic. However, many living organisms have developed several defense systems against toxic compounds, heavy metals, drugs and extraneous to the body molecules (xenobiotics). In some cases these defense systems have evolved to give rise to some enzymes able to exploit the peculiarities of some of these substances.

- It is important to know that some of these enzyme systems use alcohol to get energy. This molecule can be easily oxidized leading to high energy yields.

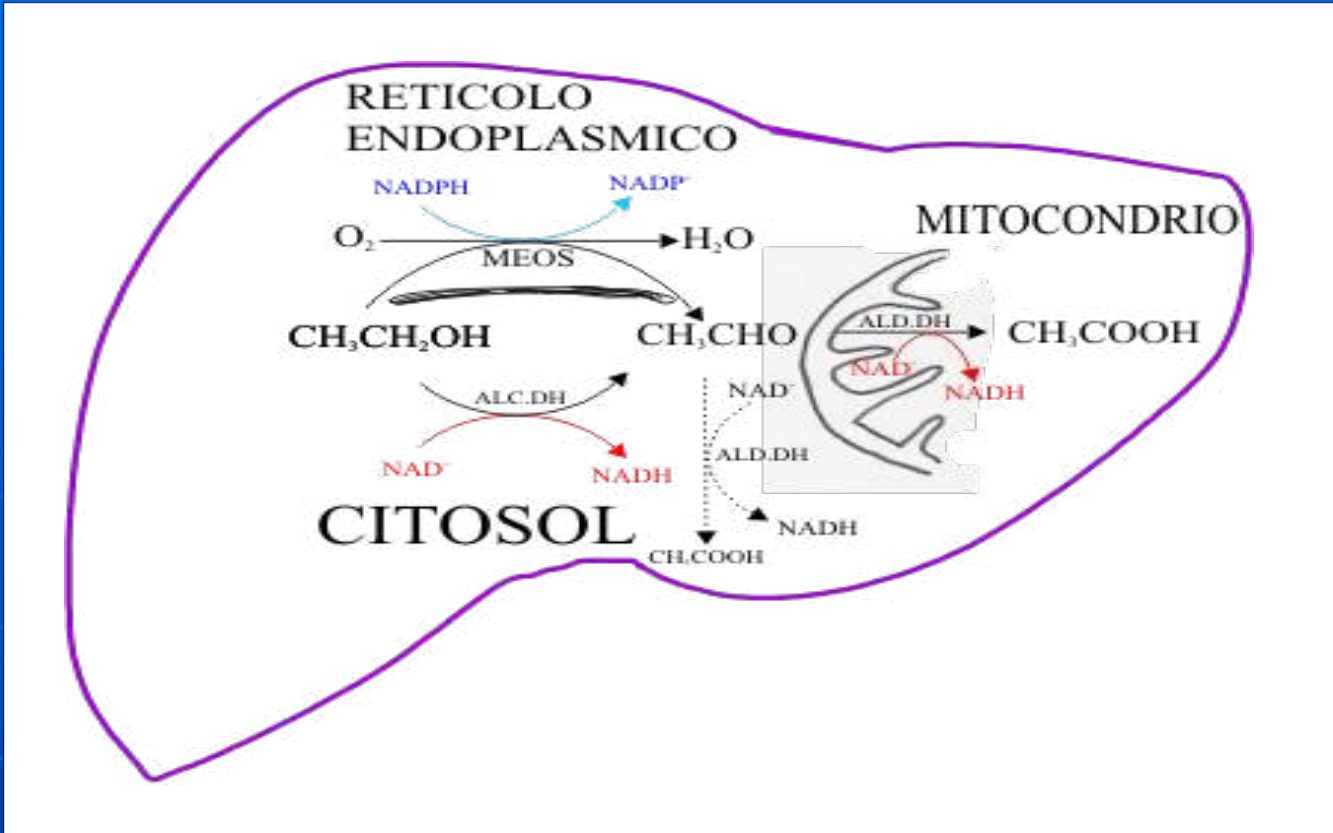
- Regarding the biochemical, nutritional and pharmacological properties of alcohol, the features of the processes and the enzyme systems that use and eliminate it in the human organism will be clearly understood

- Such processes take place mostly in the liver, that will be, accordingly, the first organ to undergo to metabolic and functional alterations by alcohol overloading, before the onset of systemic diseases derived from chronic abuse of alcohol, such as alcoholism.

Wine metabolism

- The metabolism of ethanol has several points of similarity with that of fatty acids:
- 1) oxidation of the carbon chain, with production of reduced coenzymes;
- 2) production of acetyl-CoA, which can undergo to oxidation in the Krebs cycle or exportation to other organs as soluble and diffusible molecule (acetate / ketone bodies);
- 3) ketogenic metabolism, not being able to give rise to glycidic intermediates;
- 4) request for glycolytic intermediates for the oxidation of acetyl-CoA.

Enzyme systems for ethanol oxidation in the liver



Abbreviations:MEOS = microsomal ethanol-oxidising system: microsomial system which oxidizes ethanol; ALC.DH = alcohol-dehydrogenase; ALD.DH = aldehyde-dehydrogenase.

The liver metabolizes approximately 0.12 g ethanol / hour / kg of body weight (about 8 g of alcohol / hr / 70 kg of weight).

Alcohol-dehydrogenase

- Among the four classes of alcohol-dehydrogenase, only the isoform that is present in the liver can start to oxidize the ethanol present in alcoholic drinks: in fact, the majority of alcohol ingested is absorbed within the digestive tract and reaches the liver via the portal vein.

- The liver alcohol dehydrogenase is not specific for the ethanol, being able to oxidize the methanol too. The competition for the same enzyme, together with the low concentration of methanol in wine, reduces, for this drink, the risk of toxicity from methanol and from alcohol oxidation products (aldehyde and formic acid).

- Even the parameter that measures the affinity of alcohol dehydrogenase for ethanol (K_M) is significant: the values of K_M, around 1-2 mM, is not so far from the blood alcohol values (concentration of ethanol in the blood) detectable after about an hour after administration of a standard dose of alcohol (a drink corresponds to ~12 g of ethanol).

Aldheyde-dehydrogenase

- The aldehyde-dehydrogenase enzyme shows poor specificity and is widely distributed in the body, as can be expected from an enzyme able to remove highly reactive and toxic compounds, such as the aldehydes.

- In the liver, there are two isoenzymes, differing for subcellular localization and structural and kinetic properties. The mitochondrial enzyme has higher affinity for the acetaldehyde (KM: 3 uM) with respect to the cytosolic isoform (KM around 100 uM), and lower sensibility to disulfiram inhibitor ("antabuse"). Antabuse, which is used against the abuse of alcohol, causes toxic effects due to the accumulation of acetaldehyde.

- In some populations of Far East and South-America genetic deficiencies of mitochondrial aldehyde dehydrogenase have been reported, resulting in toxic effects of alcohol intake even at low doses, probably due to a slow acetaldehyde disposal.

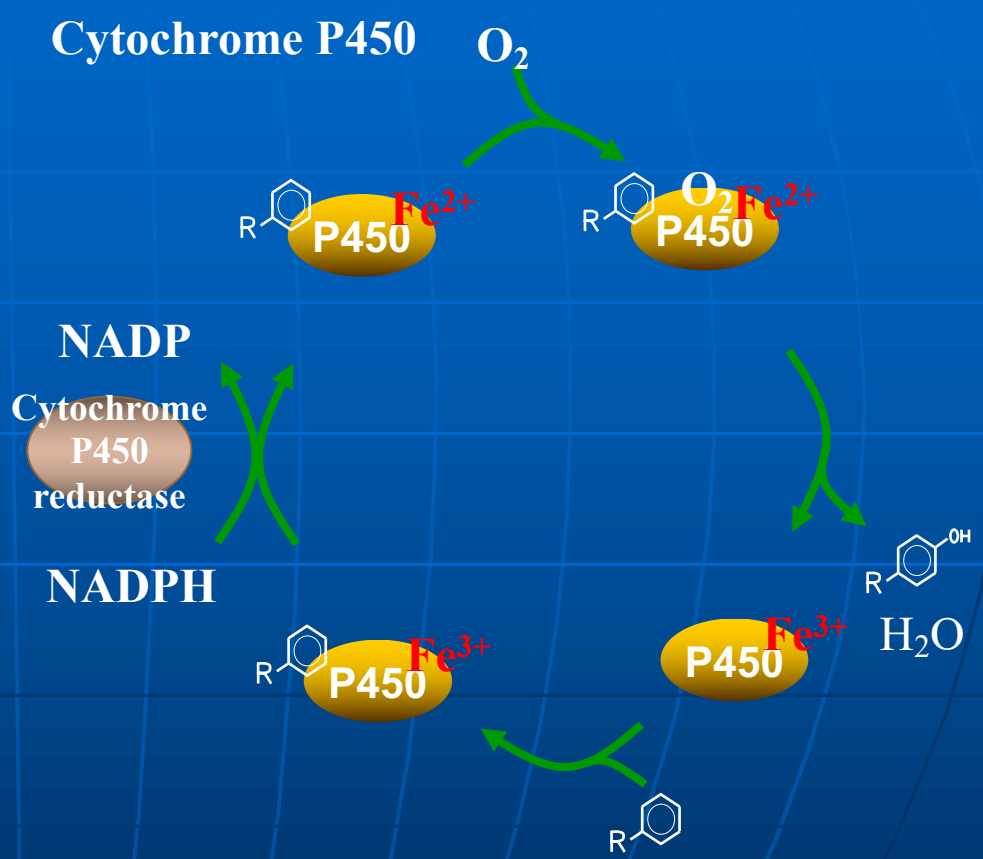
The microsomal ethanol oxidizing system (MEOS)

- The enzymatic activity of the oxydation of ethanol depends on cytochrome P450.
- The habitual consumption of alcoholic beverages promotes in the liver the induction of an enzyme system able to oxidize ethanol to acetaldehyde, using oxygen and NADPH. This alternative ethanol oxidation system is known as microsomal ethanol oxidation system (acronym: MEOS), because it is localized in the endoplasmic reticulum-system, which, during the subcellular fractionation of hepatocyte, separates as microvesicles, known as microsomes.
- When the concentration of alcohol is about or more than 4-8 mM, alcohol-dehydrogenase (Km: 1-2 mM) is presumably saturated: therefore an increase of the ethanol oxidation rate is conceivable only with an enzymatic system (the precisely MEOS) with a higher saturation limit (Km of MEOS for ethanol: about 8 mM).
- It seems that the induction of MEOS may reach an increase of 50% or even over the disposal rate of ethanol by the liver. The potential advantage derived from the induction of MEOS can actually be realized if the already complex enzymatic systems of ethanol disposal are not overloaded and disturbed by the interference of MEOS or negatively influenced by the metabolic activities related to cytochrome P450.

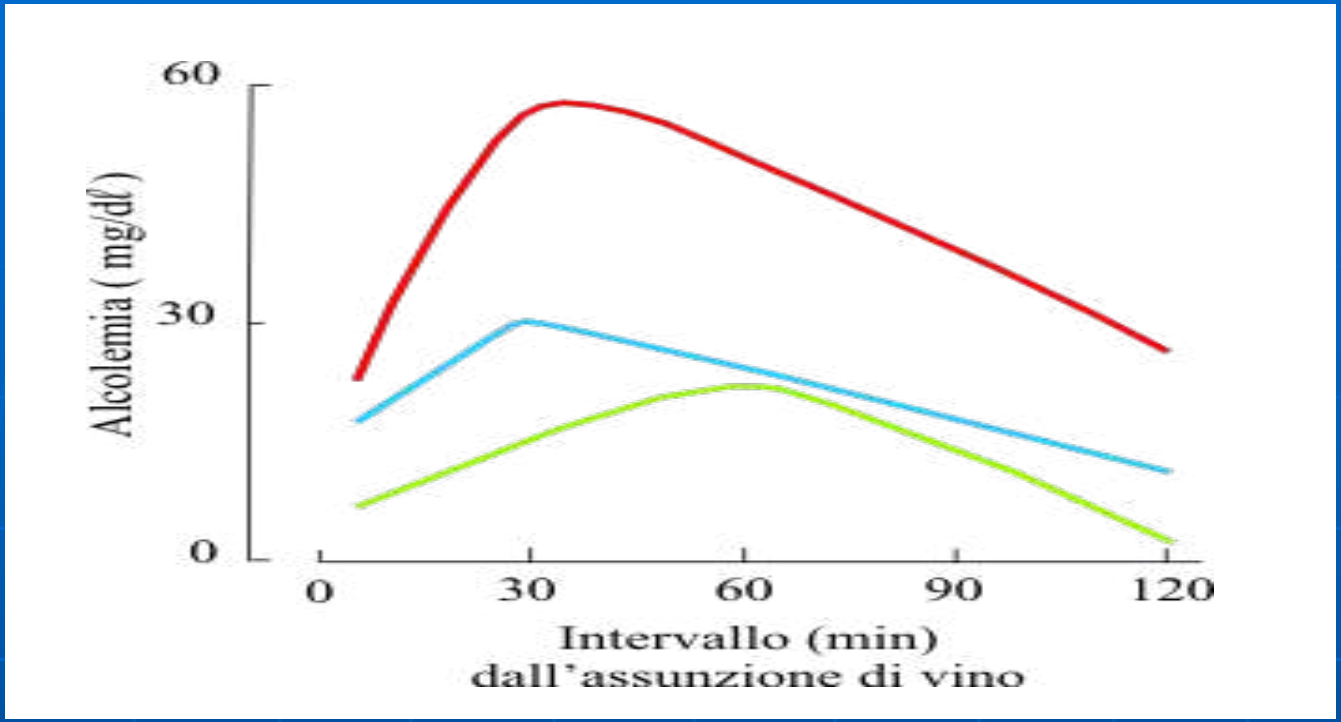
Cytochrome P450 functions

- The cytochrome P450 functions are related to the ability of bind the O_2 molecule and to oxidize a wide variety of compounds, by means of one of the two oxygen atoms, while the other is generally reduced to H_2O by the electrons of NADPH.

- These reactions are aimed at metabolic modifications of endogenous compounds (hydroxylations of steroidal compounds, hydroxylations and epoxidations of fatty acids), but also at solubilization (and subsequent elimination through the excretory systems) of xenobiotics: drugs, toxins, carcinogens and mutagens, etc..



THE CORRECT INTAKE OF ALCOHOL



Disposal of alcohol (24 g),

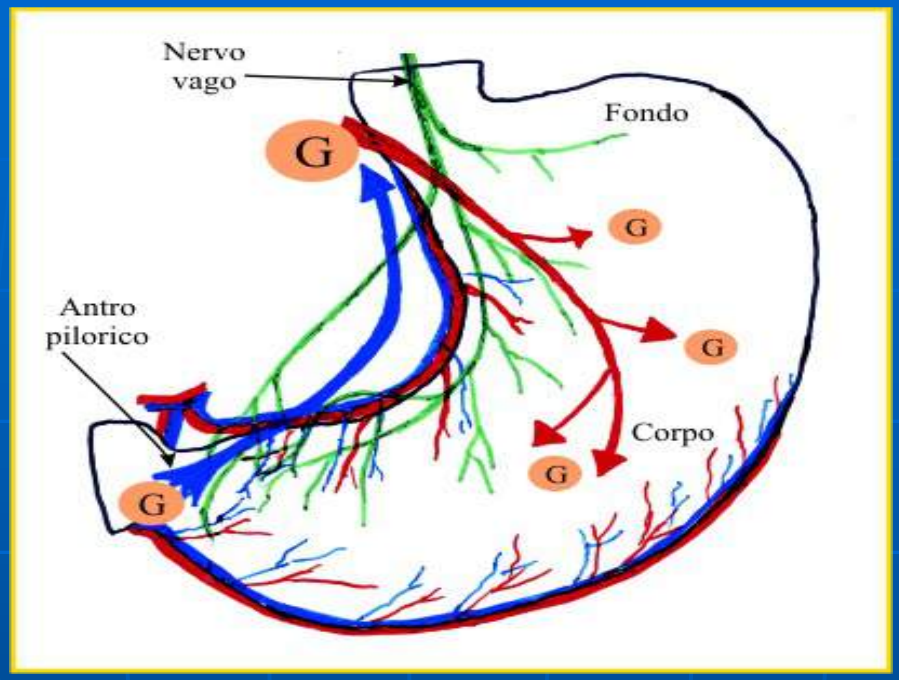
in 1/4 liter of wine at 12 ° :

-- fasting at single dose

-- during meal at single dose

-- during meal at more doses

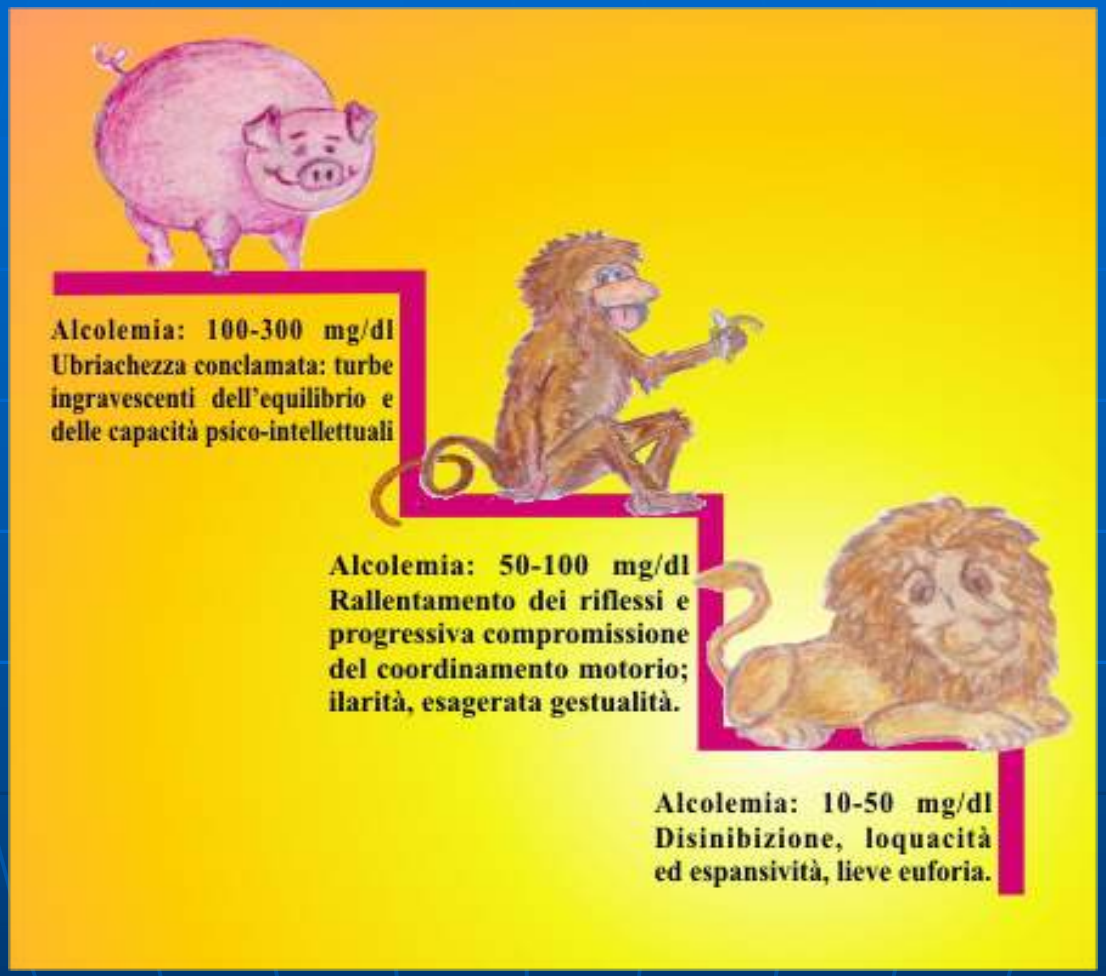
Hormonal and nervous stimulation of gastric secretion



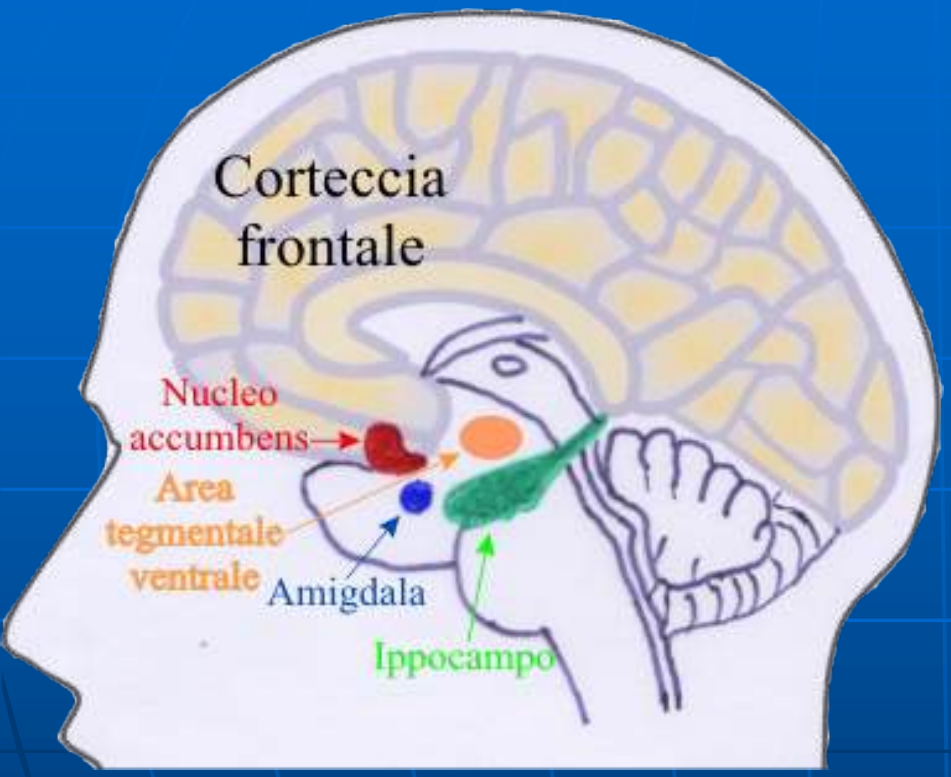
Gastrin (G), the main stomach hormone, is secreted into the blood by specific endocrine cells of the pyloric antrum, innervated by the vagus. Gastrin reaches the stomach via the blood circulation. At this site it stimulates the gastric glands to secrete hydrochloric acid and pepsin.

The secretion of gastric secretions is promoted by the vasodilator effect of ethanol, which, at not too high concentrations, activates the blood circulation supporting the digestive functions of the gastric mucosa.

Wine: the Noah drink



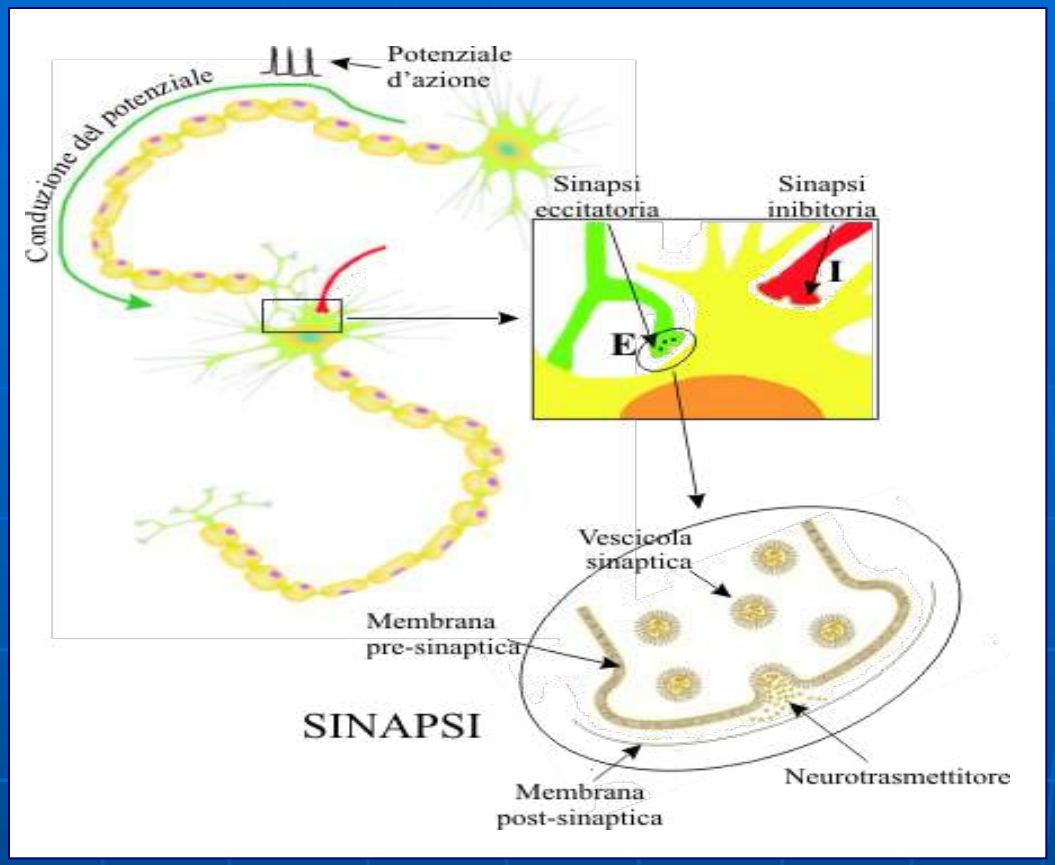
Ethanol and "gratification“ circuit



When stimulated, this circuit activates connections with other nuclei and areas of the brain (amigdala, hippocampus), which process and transmit different kind of signals to the nucleus accumbens. These signals are related to pleasure and satisfaction derived from food, sex, fun and relationships.
The frontal cortex receives and integrates the information, coordinating the behavioral response.

The brain system of pleasure and reward, probably originated in lower organisms as hunger and coupling center (to satisfy the most basic needs for survival and multiplication of the species), it has become more complex in mammals and especially in man, as a result of the connections and multiple interactions with the frontal cortex, particularly developed in humans and place of integration of the most complex neuro-psycho-behavioral interactions.

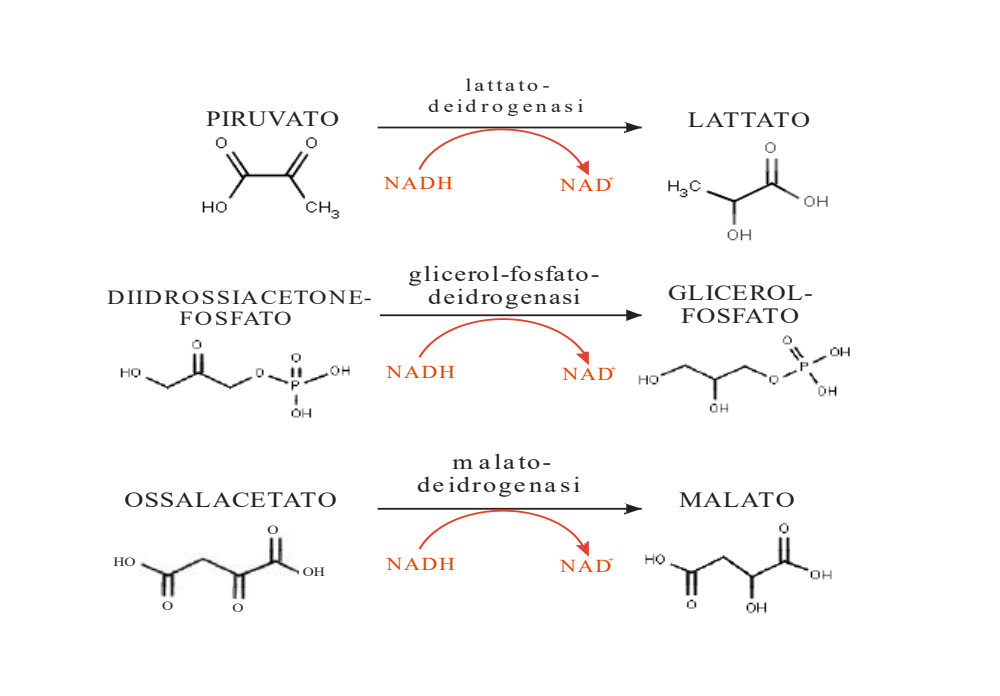
Alcohol increases the concentration of dopamine



Various researches have shown that different drugs (i.e. cocaine, nicotine, opiates and alcohol) act on the gratification circuit by increasing, with different mechanisms, the concentration of dopamine, a neuro-transmitter that propagates the signal between the neurons of this pathway. In particular, the ethanol increases the concentration of dopamine, blocking the inhibitory action on dopaminergic transmission system.

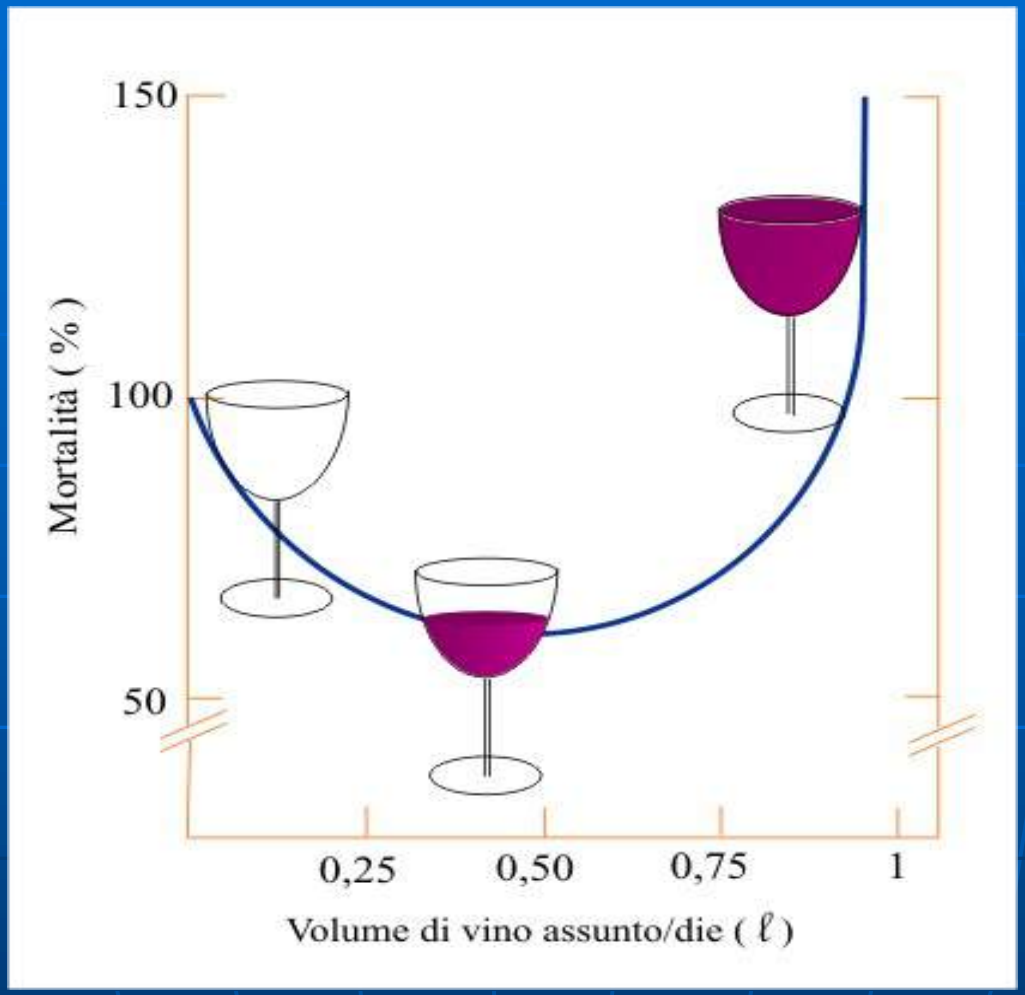
Metabolism disorders and alcohol abuse related diseases

The first and most obvious metabolic consequence of the biological oxidation of ethanol is the increase of the NADH / NAD + ratio.



Summary: **The difficulty to oxidize ethanol leads to a decrease of the oxidative metabolism of the main energy nutrients (glucides and lipids), the blockade of gluconeogenesis, the production of ketone bodies and precursors of lipid synthesis.**

BENEFITS DERIVED FROM A MODERATE ALCOHOL CONSUMPTION



Mortality in function of habitual intake of wine (J-curve).

Possible “favorable” molecular mechanisms involved in consuming small quantities of alcohol

The ischemic preconditioning conditions seem to induce the expression of different enzymes with cardioprotective activity. In fact, a brief ischemic episode seem to increase the levels of superoxide anion .O2- and nitric oxide .NO, which activate transcription factors that give rise ultimately to the expression of proteins with cardioprotective function.

The same enzymes and the same mechanism induced by the ischemic preconditioning seem to be generated by a moderate intake of ethanol.

Among the enzymes whose it has been observed an increase of expression, there are nitric oxide synthase (NOS) and superoxide dismutase (MnSOD).

The mechanism seems to be related to an increase of MnSOD levels which involve a decrease in .O2- levels and therefore a drastic reduction of its interaction with the .NO (which as mentioned above generates peroxynitrite (ONOO-), a powerful oxidant.

Consequently, the levels of .NO raise with consequent vasodilatory protective effects from ischemic events.

Possible “favorable” molecular mechanisms involved in consuming small quantities of alcohol

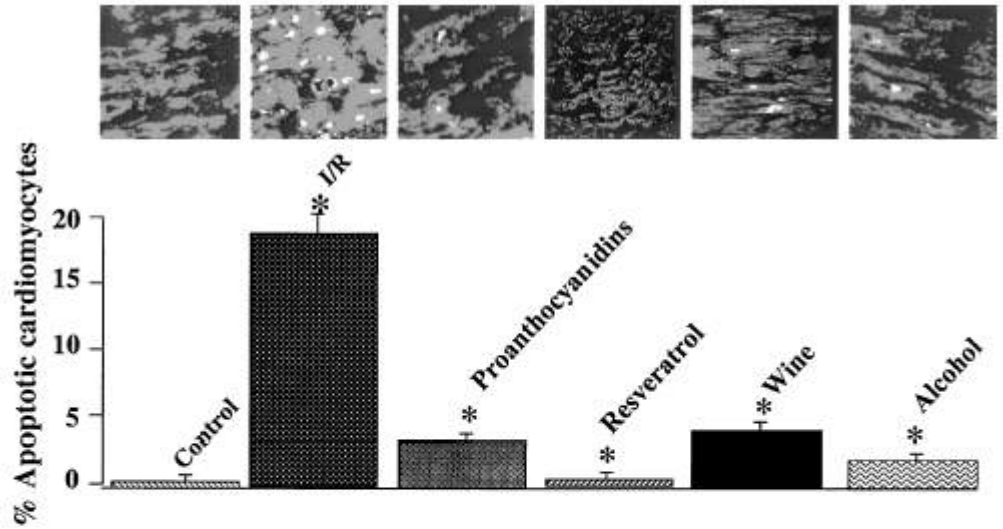
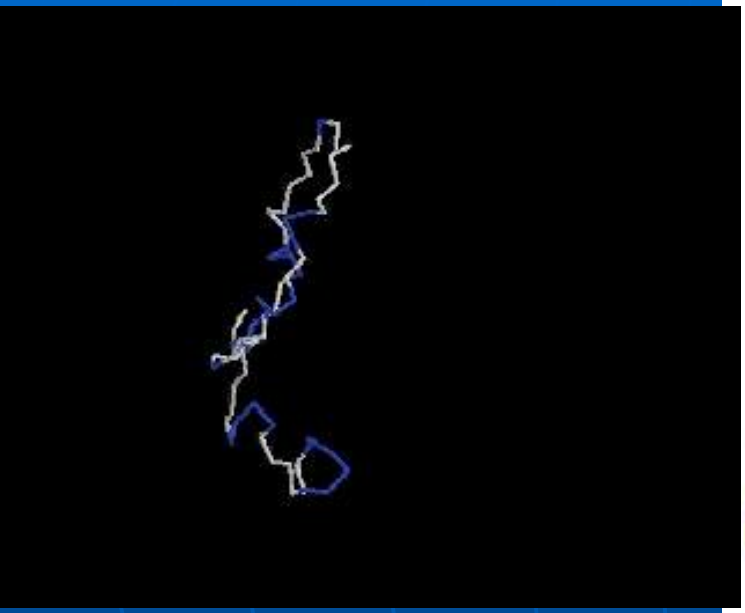
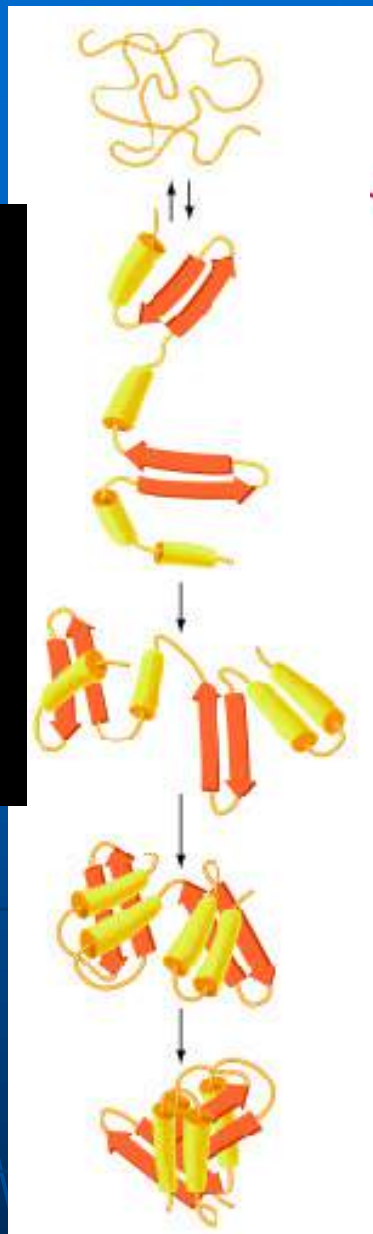


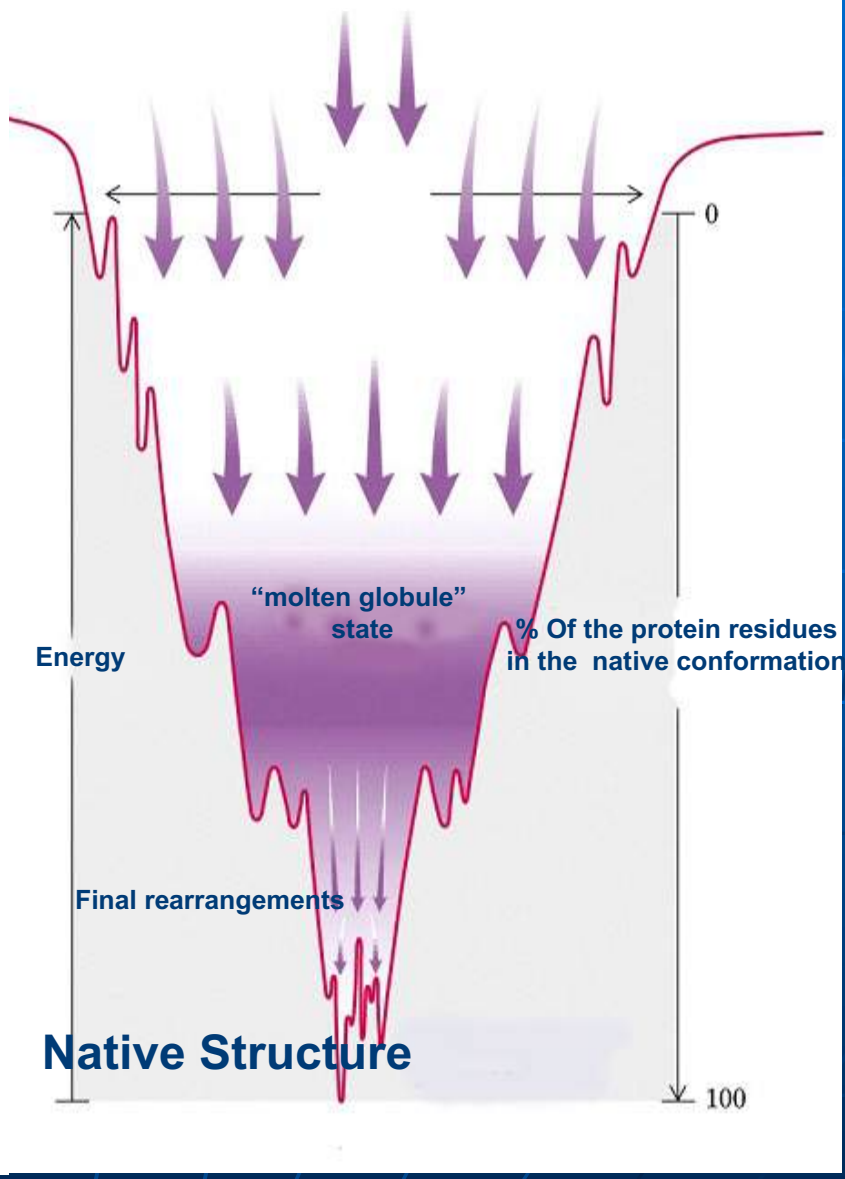
FIGURE 4. Effects of wine, its polyphenolic components, proanthocyanidin and resveratrol, and alcohol on cardiomyocyte apoptosis. Rats were fed orally with red wine extract, proanthocyanidin, resveratrol or alcohol up to a period of three weeks. At the end of three weeks, rats were anesthetized and isolated perfused working hearts were subjected to 30 min ischemia followed by two hours of reperfusion. The apoptotic cardiomyocytes were detected by Tunnel staining in conjunction with a specific antibody against α-myosin heavy chain to specifically stain the cardiomyocytes as described in the METHODS section. Representative apoptotic cells were detected by laser scanning microscopy (*top*). The results (average of at least six/group) expressed in a bar graph are shown below. * $p < 0.05$ versus I/R or control.

Protein folding



Only the native structure of a protein carries out the specific function of each protein within the cell.

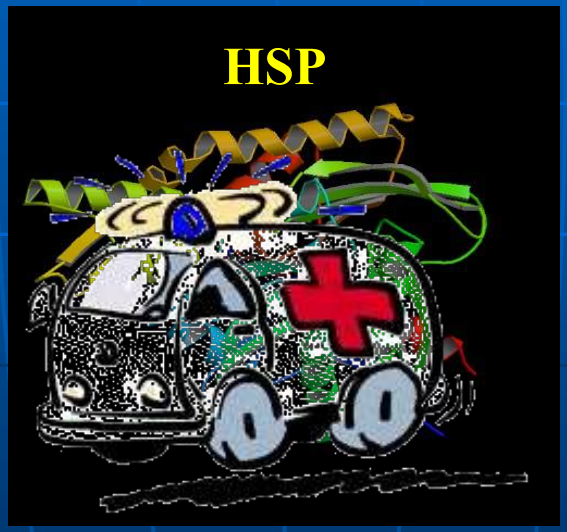




The HSPs prevent the incorrect folding of proteins

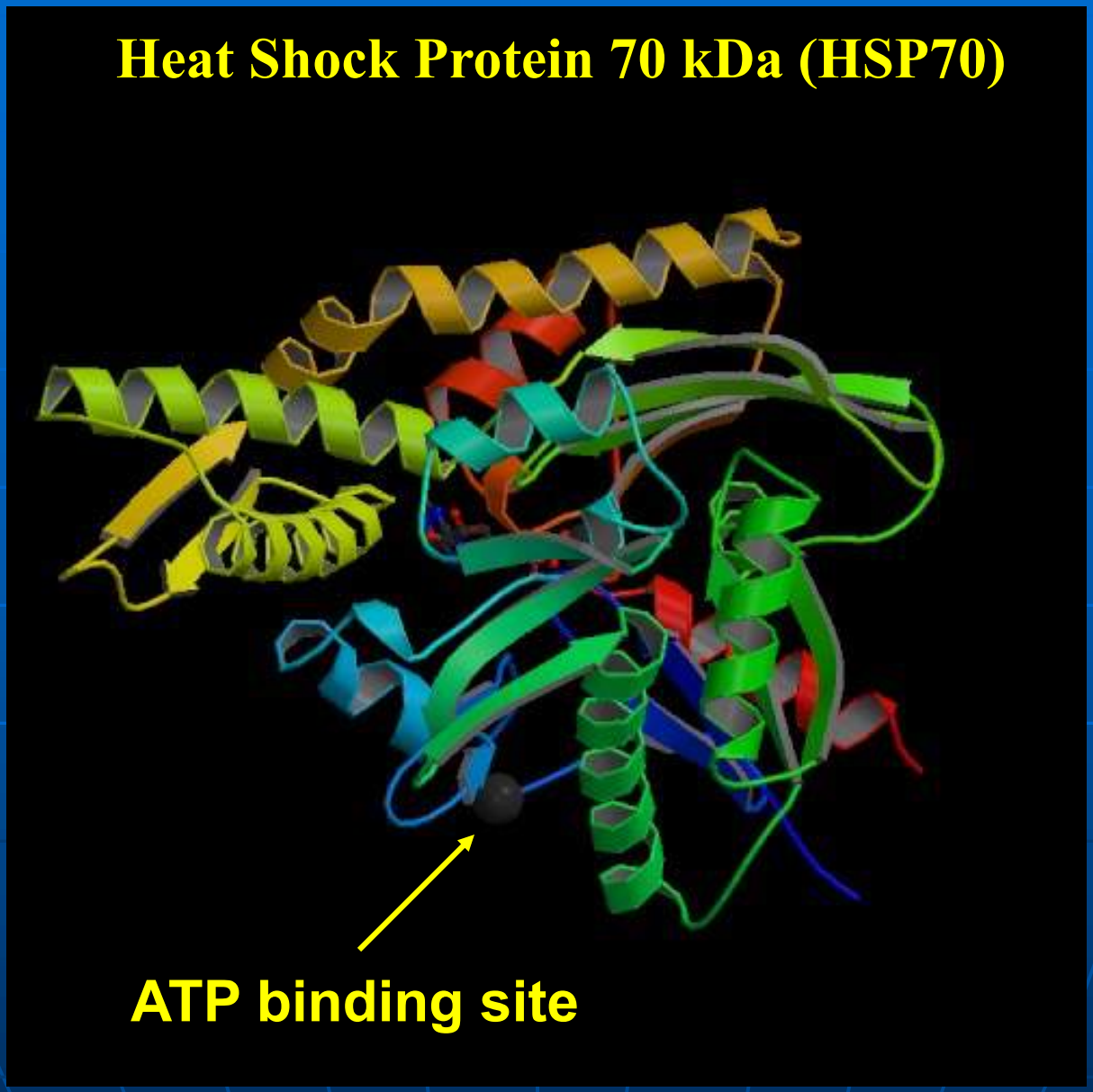
- During the biosynthesis of proteins
- During the translocation of proteins within the intracellualr compartments
- In the presence of chemical-physical stressful agents

Strongly altered hydrophobic interactions may result in the loss of the native conformation



Binding to hydropghobic residues and hydrolyzing ATP, they provide a suitable microenvironment for proper protein folding

Heat Shock Protein 70 kDa (HSP70)



Possible “favorable” molecular mechanisms involved in consuming small quantities of alcohol

Even moderate alcohol consumption induces a significant amount of oxidative stress in the heart muscle that seems to be involved in the expression of some stress-inducible proteins with cardioprotective functions.

Among these stress-inducible proteins we can find, in particular, the HSP70 and some proteins involved in the apoptosis of cardiomyocytes.

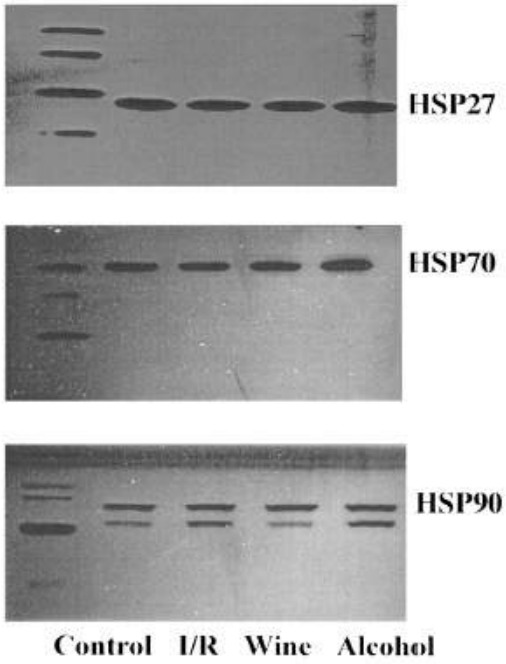
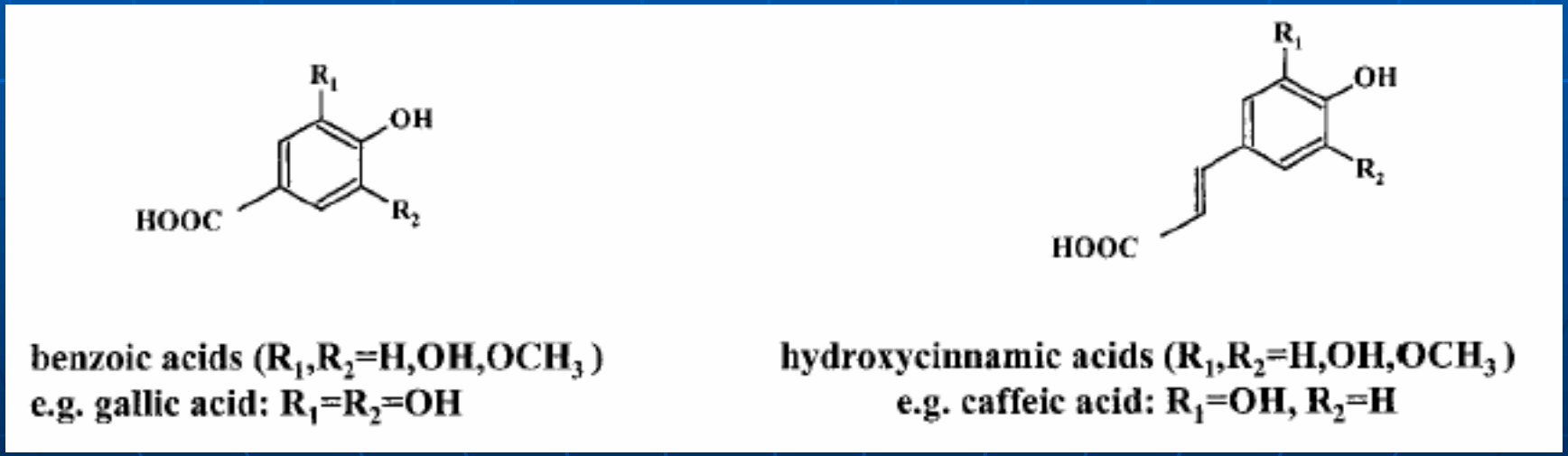


FIGURE 3. Effects of wine, its polyphenolic components, proanthocyanidin and resveratrol, and alcohol on the induction of the expression of HSP 27, HSP 70, and HSP 90 protein content of the heart. Rats were fed orally with red wine extract, proanthocyanidin, resveratrol or alcohol for three weeks. At the end of three weeks, rats were anesthetized and isolated perfused working hearts were subjected to 30 min ischemia followed by two hours of reperfusion. The HSPs was measured by Western blot analysis as described in the METHODS section. Results are representative of at least six hearts per group.

Wine, polyphenols and Nutrition

The grapevine, like other plants, produces compounds which possess antioxidant properties in vitro: the best known are simple or polymeric phenolic compounds (polyphenols). Many of these compounds are present in grapes and can be found, although in different proportions, in wine after the fermentation process. Instead, other phenolic compounds may be formed during the vinification processes; or they can be transferred from the barrels during the wine aging in wood processes. The main phenolic compounds present in wine are phenolic acids, flavonols, tannins and anthocyanins.

Phenolic acids and flavonols are present as glucosides in white and red grape peels.

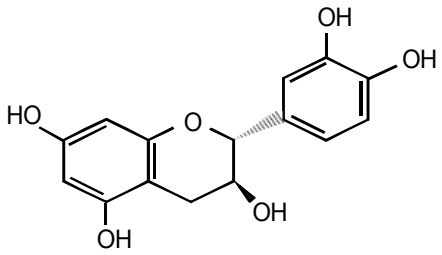


Chemical structure of benzoic and cinnamic acids, present as esters or glycosides in grape peels.

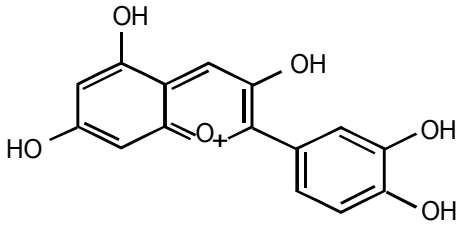
Wine, polyphenols and Nutrition

The condensed tannins are polymeric phenols, which become monomers for oxidation, releasing catechin and cyanidin.

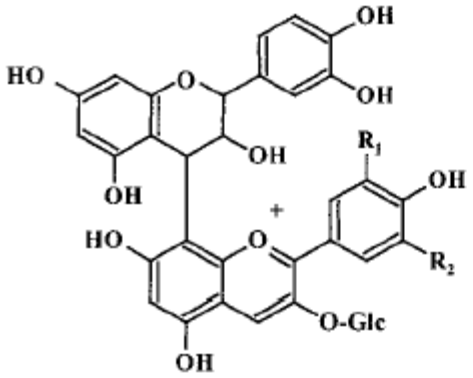
Anthocyanins are glycosides of cyanidins, present in red grape peels, which receive the color from these compounds. However, some abductees with flavanols are colorless.



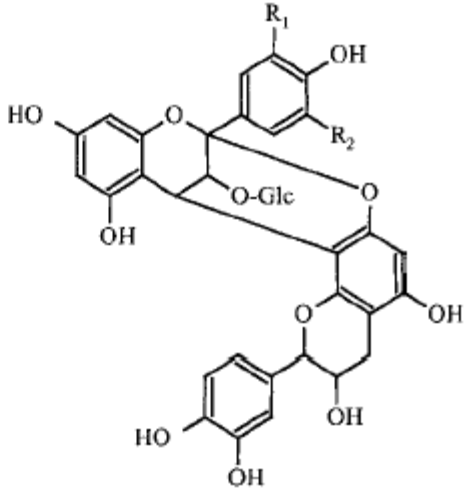
Catechina



Cianidina



flavanol-anthocyanin adduct (red pigment)

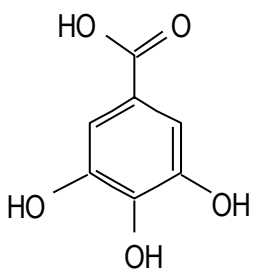


A-type anthocyanin-flavanol adduct (colorless)

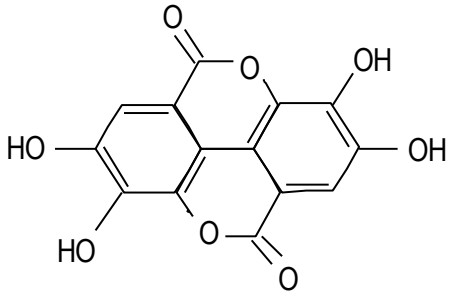
FIGURE 3. Structures of anthocyanin-flavanol dimeric adducts (R = OH or OCH_3; R' = H, OH, or OCH_3).

Wine, polyphenols and Nutrition

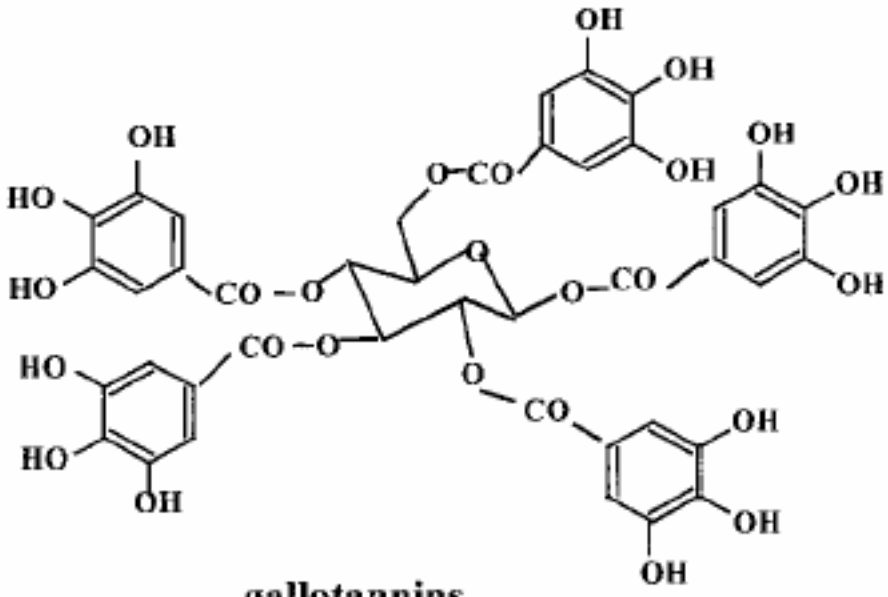
The hydrolyzable tannins (gallotannins and ellagitannins) are not present in the grapes, but are found in the wine in the form of esters, which after hydrolysis give rise to free gallic and ellagic acids.



Acido gallico



Acido ellagico

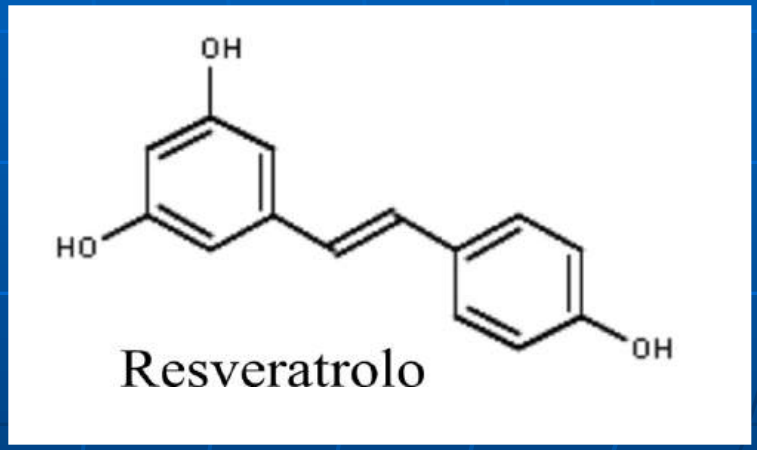


gallotannins
(e.g. pentagalloylglucose)

Wine, polyphenols and Nutrition

In wine there are varying amounts of resveratrol, a stilbene compound, derived from flavonoids, which is part of phytoalexins.

These substances are produced by the grapevine in response to biotic (attacks by microfungi) or abiotic (adverse climatic conditions, etc.) stresses and can also be formed in peels especially before the acinus maturation.



Resveratrolo

Wine, polyphenols and Nutrition

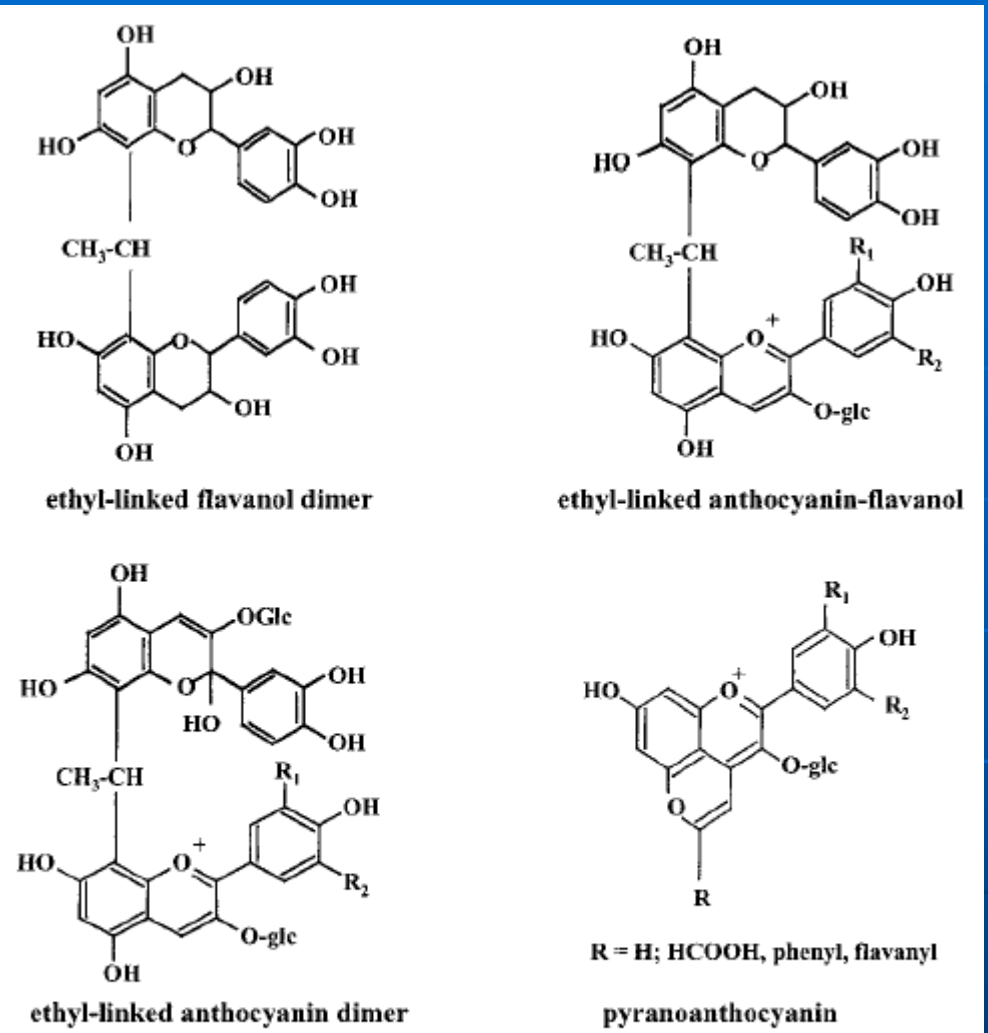


FIGURE 4. Structures of products formed in wine through reactions of tannins and/or anthocyanins with acetaldehyde and other yeast metabolites (eg, vinylphenol: R = phenyl; pyruvic acid: R = COOH) (R_1 = OH or OCH_3; R_2 = H, OH, or OCH_3).

Phenolic and polyphenolic compounds exert antioxidant activities in vitro, especially the tannins, chelating metals. In the case of flavonoids, for example, it has been demonstrated that they inhibit the lipid peroxidation and lipoxygenase in vitro. Only in the presence of reliable data about the intestinal absorption and in vivo effects of phenols and polyphenols, one can evaluate the importance of wine for health and diseases prevention.

Wine, polyphenols and Nutrition

- **Reduction of oxidative damage of plasma lipoproteins LDL.**
- **Oxidative damage is related to the content of oxidized lipids in foods that can induce an increase in the susceptibility of LDL to oxidation.**
- **The post-prandial increase of oxidized LDL (known as LDL-), in which the apo-B is partially denatured and embedded in the core of the particles, it is a valid bio-marker of oxidative stress induced by foods.**

Wine, polyphenols and Nutrition

- The wine, taken with meals, seems to minimize the post-prandial increase of lipid hydroperoxide and LDL-.
- Among the antioxidant compounds that one can find in wine, the procyanidins are those that seem to play a key role. These compounds are considered more effective than their monomers that contain catechol groups.
- Consistently with current experimental evidences it seems that the effect of wine on preventing the oxidation of post-prandial LDL is not due to a simple increase in plasma antioxidant capacity, but rather to a reduction of lipid peroxides in food.
- Indeed, the lipid peroxides contained in chylomicrons and lipoproteins seem to be the most probable effectors of the oxidative damage on circulating LDL, giving rise to an increase in LDL.

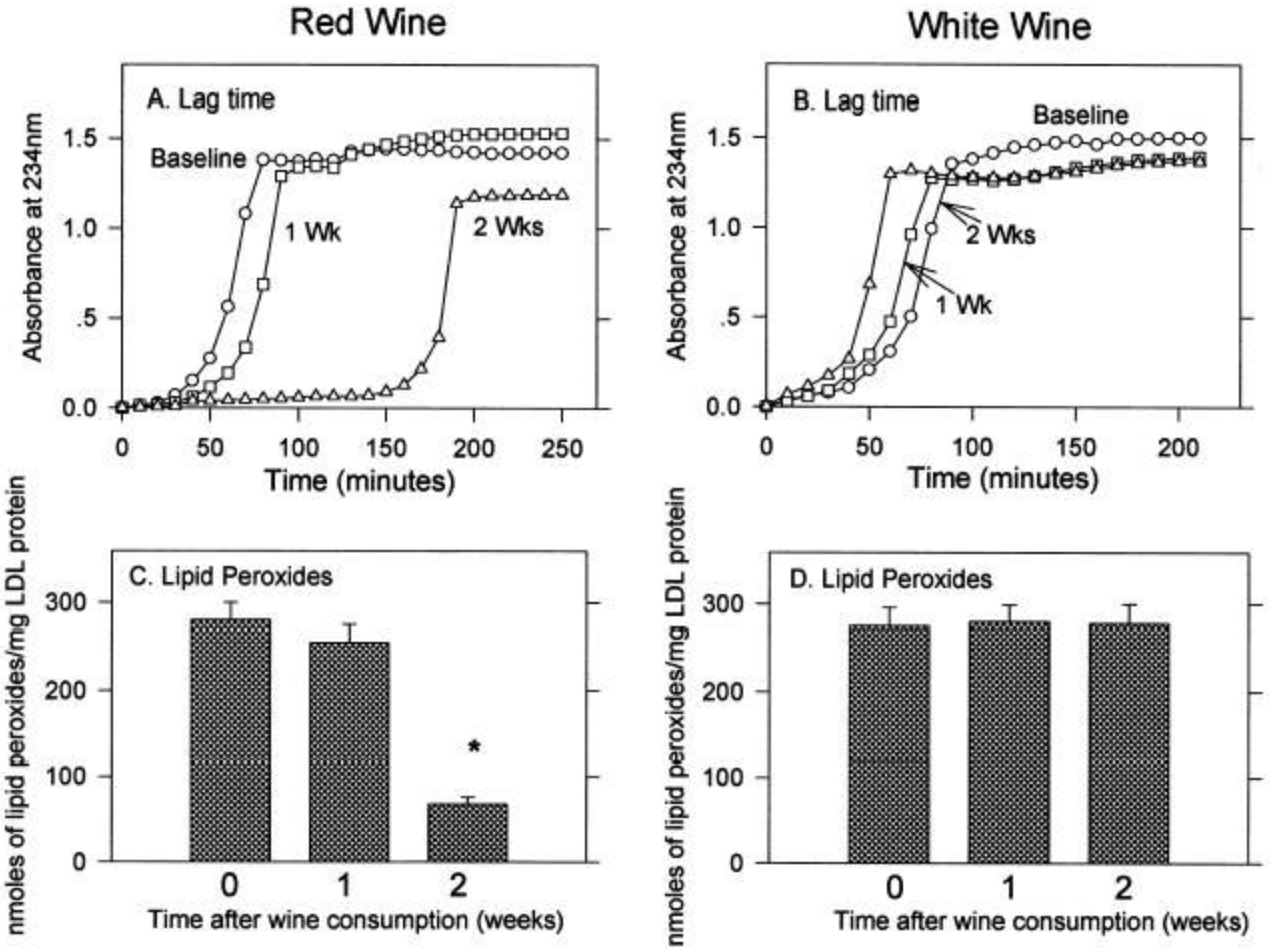


FIGURE 2. The antioxidative effects of red wine versus white wine consumption on LDL oxidation *ex vivo*. LDL (200 µg of protein/ml) isolated before ("0 time", baseline) or after one or two weeks of red wine or white wine consumption, was incubated with $CuSO_4$ (10 µmol/L). **A, B:** LDL oxidation was kinetically monitored by continuously monitoring the absorbance at 234 nm. **C, D:** LDL oxidation was determined by measuring the formation of lipid peroxides. Results are expressed as mean ± SD ($n = 3$). * $p < 0.01$ (vs. time zero).

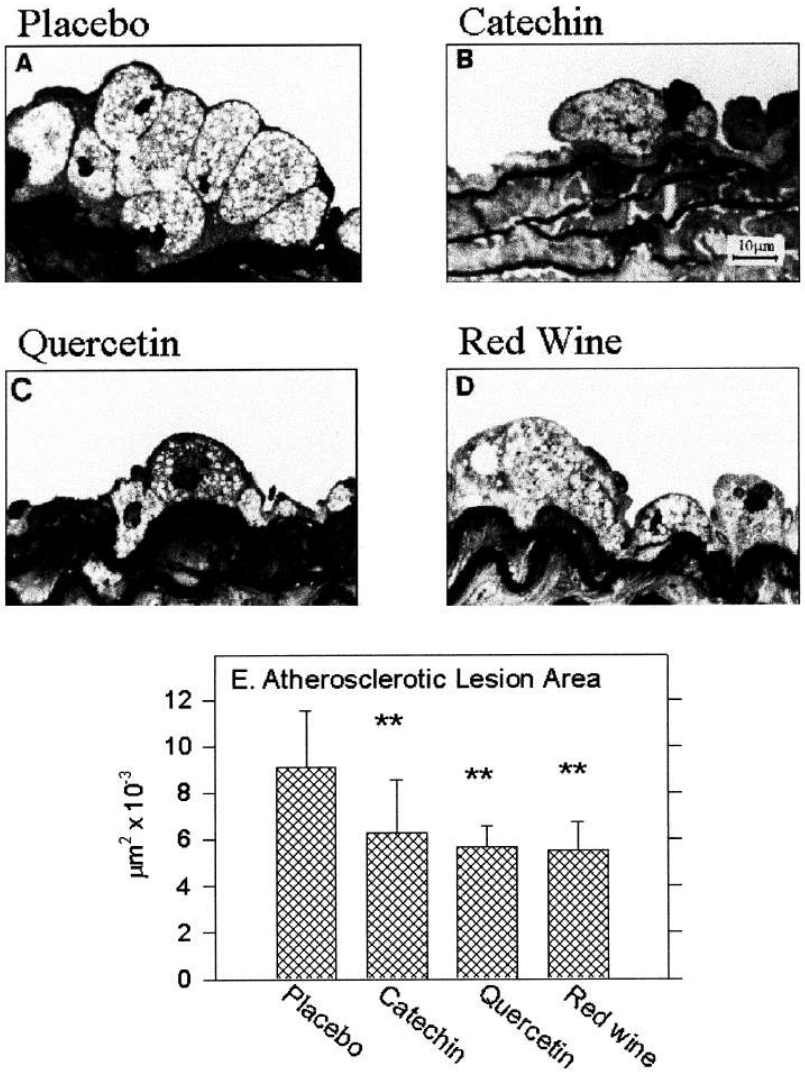


FIGURE 3. Effects of catechin, quercetin or red wine consumption by E^0 mice, on the size of their aortic arch atherosclerotic lesion area. The aortic arch derived from E^0 mice that consumed placebo, catechin, quercetin, or red wine for 6 weeks was analyzed. Photomicrographs of a typical atherosclerotic lesions of the aortic arch following treatment with placebo (**A**), catechin (**B**), quercetin (**C**), or red wine (**D**) are shown. The sessions were stained with alkaline toludine blue. All micrographs are at the same magnification. **D:** The lesion area is expressed in square micrometers ± SD. * $p < 0.05$ versus placebo.

Wine, polyphenols and Nutrition

- In vitro experiments have demonstrated that certain phenolic compounds present in wine and other alcoholic beverages, such as flavonoids, can reduce the adhesion of monocytes to endothelial cells.

- Interference with clotting mechanisms. The formation of thrombi on the surface of atherosclerotic lesions, as a result of the slowdown of the blood circulation and coagulability, is a serious and dangerous complication of atherosclerosis.

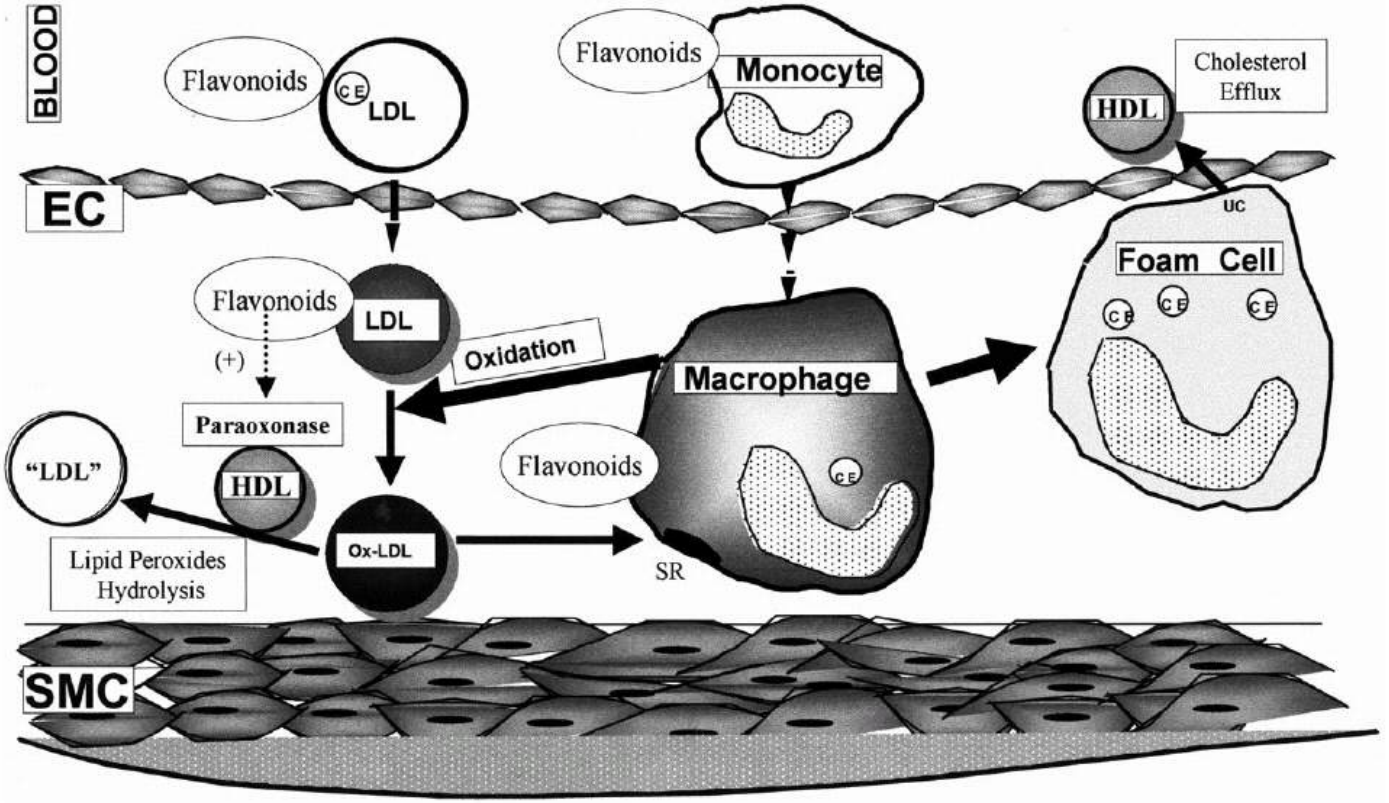


FIGURE 5. Effect of wine flavonoids on LDL oxidation and foam cell formation. Flavonoids can associate directly with LDL, resulting in the inhibition of LDL oxidation. Flavonoids can also associate with arterial cells such as monocytes/macrophages, resulting in the inhibition of macrophage-mediated oxidation of LDL. Furthermore, flavonoids preserve the activity of the enzyme paraoxonase, which further protects lipoproteins from oxidation. Altogether, these effects lead to a reduced formation of macrophage-foam cells, and thus attenuate the development atherosclerotic lesion.

Table 1: Characteristics of the subjects of the single-dose and the dietary intervention trial

	Single-dose analysis			Dietary intervention trial		
Group	RW	DRW	Controls	RW	DRW	Controls
Subjects (f/m), *n*	9 (7/2)	9 (8/1)	9 (6/3)	24 (12/12)	25 (15/10)	25 (15/10)
Age, *years*	27.1 ± 9.0[a]	26.2 ± 3.5[b]	31.4 ± 5.8[a,b]	30.0 ± 8.3	26.7 ± 6.4	28.8 ± 7.1
BMI kg/m^2	20.8 ± 1.1	21.8 ± 1.9	22.0 ± 2.8	23.5 ± 3.3[c,d]	21.9 ± 2.1[c]	21.4 ± 2.2[d]
Exercise *h/week*	3.4 ± 2.9	2.7 ± 3.3	1.9 ± 3.3	2.2 ± 2.2	2.0 ± 2.0	2.9 ± 2.4

RW: Red wine; DRW: dealcoholized red wine; f: female, m: male
Values given are means ± SD. Values marked with identical letters differ, $p \leq 0.05$ by Mann-Whitney U-test

Table 3: Antioxidant parameters in healthy volunteers after single ingestion of native or dealcoholized red wine

	RW	DRW	Controls
Parameter			
TPP, *mg CE/L*			
Baseline	15.1 ± 2.0	15.9 ± 2.0	13.9 ± 2.8
90 min	17.7 ± 1.8*[a]	18.3 ± 2.5*[b]	14.3 ± 3.5[a,b]
360 min	15.5 ± 1.8*	16.6 ± 2.0*	14.3 ± 2.7*
TEAC, *mmol/L*			
Baseline	1.22 ± 0.08	1.25 ± 0.08	1.23 ± 0.05
90 min	1.25 ± 0.07	1.28 ± 0.08	1.26 ± 0.05
360 min	1.23 ± 0.08	1.24 ± 0.07	1.24 ± 0.04
Vitamin C, *mg/dL*			
Baseline	1.33 ± 0.26	1.15 ± 0.18	1.14 ± 0.30
90 min	1.27 ± 0.21	1.18 ± 0.18	1.17 ± 0.35
360 min	1.11 ± 0.22*	1.35 ± 0.32*	1.14 ± 0.26
Uric acid, *mg/dL*			
Baseline	4.6 ± 1.0	4.8 ± 1.2	4.6 ± 1.1
90 min	5.3 ± 1.0*	4.9 ± 1.1	4.9 ± 1.2
360 min	4.5 ± 0.9	4.5 ± 1.0	4.4 ± 1.1
Albumin, *g/dL*			
Baseline	4.1 ± 0.4	4.4 ± 0.4	4.1 ± 0.4
90 min	4.4 ± 0.4	4.4 ± 0.5	4.3 ± 0.3*
360 min	4.4 ± 0.4	4.4 ± 0.5	4.4 ± 0.5
Bilirubin, *mg/dL*			
Baseline	0.73 ± 0.44	0.69 ± 0.27	0.54 ± 0.23
90 min	0.71 ± 0.37	0.68 ± 0.22	0.61 ± 0.27*
360 min	0.52 ± 0.29*	0.53 ± 0.26*	0.50 ± 0.22
TM_0, *arbitrary units*			
Baseline	1,86 ± 0,48	1,98 ± 0,33	2,19 ± 0,67
90 min	2,03 ± 0,43	2,36 ± 0,23	2,43 ± 0,43
360 min	2,61 ± 0,43*	2,67 ± 0,24*	2,33 ± 0,44
TM_{300}, *arbitrary units*			
Baseline	1,69 ± 0,92	1,43 ± 0,78	1,15 ± 1,07
90 min	1,39 ± 0,65	1,05 ± 0,87	0,78 ± 1,23
360 min	1,22 ± 0,67[c]	0,21 ± 0,45*[c,d]	1,21 ± 0,52[d]

Amounts of study drinks ingested were 200 mL red wine (RW), 175 mL dealcoholized red wine (DRW) or 200 mL water (Controls)
TPP: total phenolic content in plasma; CE: catechin equivalents; TEAC: trolox equivalent antioxidant capacity; TM_0: Tail Moment in untreated cells (endogenous DNA strand breaks);
TM_{300}: Tail Moment in cells treated with 300 μM H_2O_2 for 20 min (exogenous DNA strand breaks)
Values are means ± SD, n = 9 for all experiments except for control group at 90 min, n = 7 for all parameters; DRW, n = 8 for TM_0, TM_{300}, and control group at 360 min, n = 6 for TM_0, TM_{300}
*Values different from baseline, $P < 0.05$ by Wilcoxon signed rank test
[a-d]Values marked with identical letters differ between groups, $P < 0.05$ by Mann-Whitney U-test

Table 2: Polyphenol intake from a single serving of red wine or dealcoholized red wine

	RW	DRW
Serving, *mL*	200	175
Total phenolics,[3] *mg CE*	293.2	271.6
TEAC, *mmol/L*	3.8	2.7
Phenolic acids		
Gallic acid, *mg*	8.0	9.4
Caffeic acid,[1] *mg*	3.7	3.1
p-Coumaric acid,[2] *mg*	0.7	0.8
Flavonoids		
Catechin, *mg*	26.5	10.8
Epicatechin, *mg*	14.4	8.5
Malvidin, *mg*	8.5	4.7
Peonidin, *mg*	1.0	0.5

RW: Red wine; DRW: dealcoholized red wine; CE: catechin equivalents; TEAC: trolox equivalent antioxidant capacity
[1]calculated from caftaric acid
[2]calculated from p-coumaroyl-glucosyl-tartrate
[3]Folin method

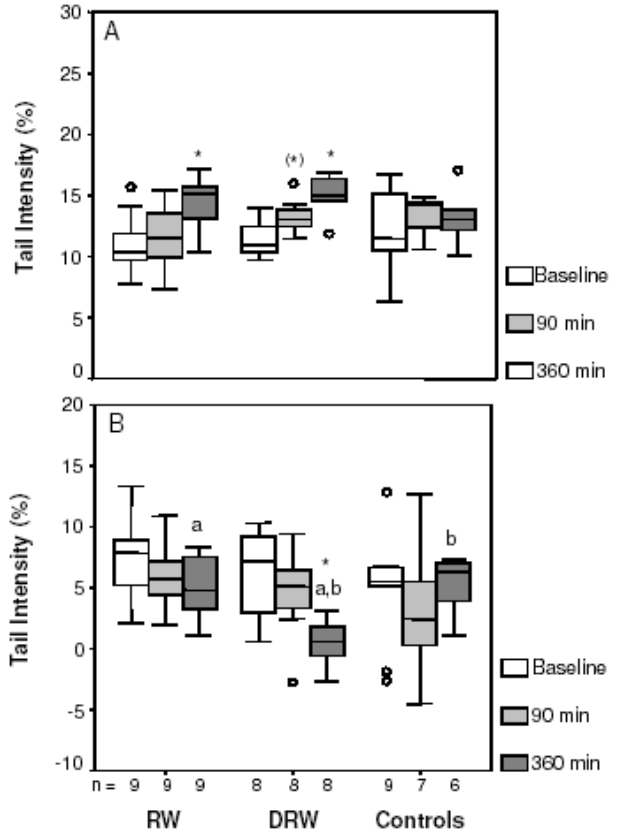


Figure 1
DNA strand breaks in peripheral leukocytes after single ingestion of native or dealcoholized red wine. A) Tail Intensity in untreated cells (endogenous DNA strand breaks) B) Tail Intensity in cells treated with 300 μM H_2O_2 for 20 min (exogenous DNA strand breaks)Amounts of study drinks ingested were 200 mL red wine (RW), 175 mL dealcoholized red wine (DRW) or 200 mL water (Controls) The box represents the distribution falling between the 25th and 75th percentiles, with the median as the horizontal line within the box. The whiskers connect the largest and smallest values not categorized as outliers or extreme values, which are represented by single data points. *, ** Values different from baseline, *P < 0.05; ** P < 0.01 by Wilcoxon signed rank test [a,b]Values marked with identical letters differ between groups, P < 0.01 by Mann-Whitney U-test

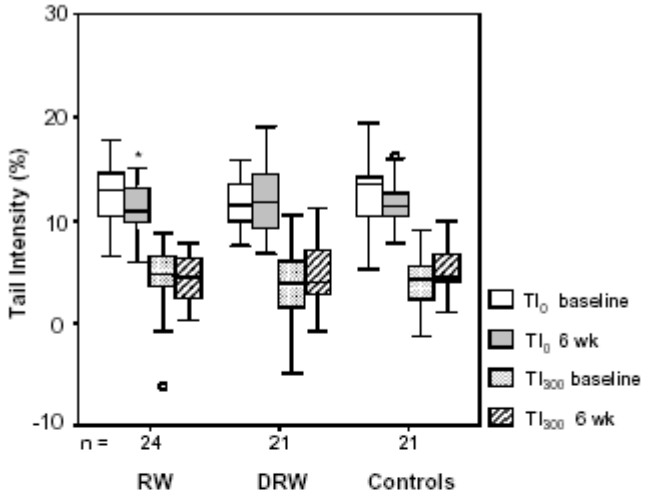


Figure 2
DNA strand breaks in peripheral leukocytes after regular consumption of native or dealcoholized red wine. Amounts ingested daily for 6 weeks were 200 mL red wine (RW) or 175 mL dealcoholized red wine (DRW). Control subjects did not receive any study drink. TI_0: Tail Intensity in untreated cells (endogenous DNA strand breaks); TI_{300}: Tail Intensity in cells treated with 300 μM H_2O_2 for 20 min (exogenous DNA strand breaks)Boxes represent the distribution falling between the 25th and 75th percentiles, with the median as the horizontal line within the box. The whiskers connect the largest and smallest values not categorized as outliers or extreme values, which are represented by single data points. *Value different from baseline, P < 0.05 by Wilcoxon signed rank test.

Table 4: Antioxidant parameters in healthy volunteers after regular consumption of native or dealcoholized red wine

	RW	DRW	Controls
Subjects, *n*	24	25	25
Parameter			
TPP, *mg CE/L*			
Baseline	15.6 ± 1.6	15.6 ± 2.1	15.5 ± 1.4
6 wk	16.4 ± 1.4*	16.0 ± 2.0	15.3 ± 2.2
TEAC, *mmol/L*			
Baseline	1.47 ± 0.06	1.44 ± 0.08	1.46 ± 0.08
6 wk	1.45 ± 0.04	1.42 ± 0.07	1.43 ± 0.07
Vitamin C, *mg/dL*			
Baseline	1.30 ± 0.28	1.43 ± 0.36	1.48 ± 0.29
6 wk	1.35 ± 0.26	1.42 ± 0.39	1.57 ± 0.28
α-Tocopherol, *mg/dL*			
Baseline	11.3 ± 3.0	11.2 ± 2.6	11.2 ± 2.9
6 wk	11.3 ± 3.0	11.5 ± 2.7	11.0 ± 2.7
Uric acid, *mg/dL*			
Baseline	4.7 ± 1.2	4.4 ± 1.1	4.4 ± 1.2
6 wk	4.9 ± 1.2	4.5 ± 0.9	4.7 ± 1.5*
Albumin, *g/dL*			
Baseline	4.2 ± 0.4	4.3 ± 0.4	4.2 ± 0.4
6 wk	4.3 ± 0.4	4.1 ± 0.6	4.2 ± 0.5
Bilirubin, *mg/dL*			
Baseline	0.68 ± 0.28	0.68 ± 0.33	0.64 ± 0.33
6 wk	0.61 ± 0.35	0.59 ± 0.27*	0.62 ± 0.35
TM_0, *arbitrary units*			
Baseline	2,18 ± 0,56	1,97 ± 0,56	2,22 ± 0,79
6 wk	1,88 ± 0,48*	2,05 ± 0,70	1,95 ± 0,49
TM_{300}, *arbitrary units*			
Baseline	1,02 ± 0,69	0,87 ± 0,72	0,95 ± 0,54
6 wk	0,96 ± 0,49	0,92 ± 0,65	1,18 ± 0,50

Amounts ingested daily for 6 weeks were 200 mL red wine (RW) or 175 mL dealcoholized red wine (DRW). Control subjects did not receive any study drink.

TPP: total phenolic content in plasma; CE: catechin equivalents; TEAC: trolox equivalent antioxidant capacity; TM_0: Tail Moment in untreated cells (endogenous DNA strand breaks);

TM_{300}: Tail Moment in cells treated with 300 μM H_2O_2 for 20 min (exogenous DNA strand breaks)

Values are means ± SD

*Values different from baseline, $P < 0.05$ by Wilcoxon signed rank test

Cultivar	Number of samples	Main area of cultivation (Italy)	Area in production (Ha)	Average berry weight (g)[b]	Percent skins by weight[c]	Percent seeds by weight[c]	Ripening[d]	Productivity[e]	Dimension of the cluster[f]	Soluble solids (°Brix) [g]	Acidity (tartaric acid, g/L) [g]	Production of wines
Sagrantino[a]	3	Central (Umbria)	161	1.5	24.6	4.8	L	2	1.5–2	H	M	aged
Tamurro	1	South (Basilicata)	<500	2.3	20.0	2.0	M L	2	1.5	M	L	young
Groppello gentile	1	North (Lombardia)	493	2.4	17.5	3.0	M L	2.5	2.5	M	M	young
Lagrein[a]	3	North (Trentino Alto Adige)	374	2.4	17.7	4.3	L	2.5	2.5	M	M H	young to aged
Rebo	3	North (Trentino)	34	2.1	15.7	5.1	M L	2	2	M H	M	young to aged
Teroldego[a]	23	North (Trentino)	505	2.1	16.6	4.7	M L	2.5	2	M H	M	young to aged
Marzemino[a]	7	North (Trentino, Lombardia)	895	2.1	17.2	5.5	M E	2.5	1.5–3	M H	M	young to aged
Enantio[a]	3	North (Trentino)	2,178	1.5	20.6	4.7	L	2.5	2.5	M L	M H	young
Croatina	3	North West (Lombardia, Piemonte)	4,486	1.8	23.0	2.8	L	3[i]	1.5–3	M	L	aged
Schiava[k]	3	North (Trentino, Lombardia)	1,196	4.0	18.2	3.2	L	3[i]	1.5–3	M	M L	young
Lambrusco salamino	2	North (Emilia, Lombardia)	4,677	1.2	21.6	5.8	M L	2.5	2.5	M L	M H	young

Cultivar	Number of samples	Main area of cultivation (Italy)	Area in production (Ha)	Average berry weight (g)[b]	Percent skins by weight[c]	Percent seeds by weight[c]	Ripening[d]	Productivity[e]	Dimension of the cluster[f]	Soluble solids (°Brix) [g]	Acidity (tartaric acid, g/L) [g]	Production of wines
Nebbiolo	5	North West (Lombardia, Piemonte)	5,246	2.0	17.6	4.3	L	2.5	2.5	M H	M	aged
Malvasia nera di Lecce	3	South (Puglia)	2,435	2.3	20.9	4.2	M L	2.5	2.5	M H	M H	young
Aglianico[i]	3	South (Campania, Basilicata, Puglia)	13,042	2.1	18.0	4.9	L	2	1.5–2''	M H	M H	aged
Calabrese	2	South Islands (Sicilia)	14,182[h]	2.1	25.5	2.8	M L	2.5	1.5	H	M L	young to aged
Nero d'Avola	2	South Islands (Sicilia)	14,182[h]	2.0	24.7	2.7	M L	2.5	1–2.5	H	M L	young to aged
Primitivo	3	South (Puglia)	17,249	1.9	22.9	3.0	E	2.5	1.5	H	L	young to aged
Montepulciano	3	Central and South (Marche, Abruzzo, Puglia, Sicilia)	31,008	2.6	21.3	2.6	L	3	2.5	M H	M	young to aged
Negroamaro	3	South (Puglia)	31387	2.7	17.8	3.7	L	2.5	2	H	M	young to aged
Barbera	3	North West (Lombardia, Piemonte)	47,120	2.3	17.5	3.3	L	2.5	1–2.5	M H	H	young to aged
Sangiovese	3	Central and North (Toscana, Marche, Umbria, Emilia, Sicilia, Puglia)	86,196	2.4	21.5	4.6	M L	3	1–2.5	M H	M	young to aged
Merlot[a]	9	North Central South	31,872 (145,000)[i]	1.7	22.4	4.2	L	2.5	1.5–2.5	H	M L	young to aged
Pinot noir[a]	3	North (Trentino Alto Adige, Veneto, Friuli)	8,538 (37,000)[i]	1.8	20.3	4.5	E	1–1.5	1.5	M H	M	young to aged
Cabernet Sauvignon[a]	25	North Central South	2403 (140,000)[i]	1.6	23.1	4.5	L	2.5	2.5	H	M L	young to aged
Syrah[a]	3	North Central South	102 (35,000)[i]	2.0	17.2	4.0	M L	2.5	2.5	M H	M L	young to aged

TABLE 3. Mean content of extractable polyphenols (Folin Ciocalteu) in different cultivars of red grape[a]

Range	Cultivar
$1{,}500 \leq X < 1{,}800$	Schiava–Barbera–Syrah
$1{,}800 \leq X < 2{,}100$	Negroamaro–Nebbiolo–Cabernet Sauvignon
$2{,}100 \leq X < 2{,}400$	Calabrese–Groppello–Marzemino–Malvasia nera di Lecce–Rebo–Merlot–Sangiovese–Enantio
$2{,}400 \leq X < 2{,}700$	Montepulciano–Nero d'Avola–Tamurro
$2{,}700 \leq X < 3{,}000$	Primitivo–Lagrein–Teroldego
$3{,}000 \leq X < 3{,}300$	Lambrusco salamino–Pinot noir
$3{,}300 \leq X < 3{,}600$	Croatina
$3{,}600 \leq X < 3{,}900$	Aglianico
$3{,}900 \leq X < 4{,}200$	Sagrantino

[a]Data as (+)-catechin, mg/kg of grape.

TABLE 4. Mean content of extractable anthocyanins in different cultivars of red grape[a]

Range	Cultivar
$300 \leq X < 500$	Primitivo–Schiava–Nebbiolo–Pinot noir
$500 \leq X < 700$	Sangiovese–Negroamaro–Nero d'Avola–Tamurro–Calabrese–Malvasia nera di Lecce–Groppello
$700 \leq X < 900$	Sagrantino–Aglianico–Lambrusco salamino–Cabernet Sauvignon–Barbera
$900 \leq X < 1,100$	Syrah–Merlot–Montepulciano
$1,100 \leq X < 1,300$	Croatina–Marzemino–Rebo
$1,300 \leq X < 1,500$	Enantio
$1,500 \leq X < 1,700$	Lagrein
$1,700 \leq X < 1,900$	Teroldego

[a]Data as malvidin 3-monoglucoside chloride, mg/kg of grape.

TABLE 5. Mean content of extractable proanthocyanidins (PR) in different cultivars of red grape[a]

Range	Cultivar
1,600 ≤ X < 1,900	Schiava–Syrah–Barbera
1,900 ≤ X < 2,200	Calabrese–Negroamaro
2,200 ≤ X < 2,500	Marzemino–Groppello–Rebo
2,500 ≤ X < 2,800	Merlot–Sangiovese–Cabernet Sauvignon–Malvasia nera di Lecce
2,800 ≤ X < 3,100	Nebbiolo–Nero d'Avola–Enantio–Teroldego–Primitivo–Pinot noir–Montepulciano
3,100 ≤ X < 3,400	Lagrein
3,400 ≤ X < 3,700	Tamurro–Aglianico
3,700 ≤ X < 4,000	Lambrusco salamino–Croatina
4,000 ≤ X < 4,300	Sagrantino

[a]Data as cyanidin, mg/kg of grape.

TABLE 6. Mean content of extractable catechins and proanthocyanidins, reactive to vanillin (VAN), in different cultivars of red grape[a]

Range	Cultivar
$800 \leq X < 1200$	Barbera–Syrah
$1,200 \leq X < 1,600$	Marzemino–Enantio–Schiava–Negroamaro–Malvasia nera di Lecce–Rebo–Cabernet Sauvignon–Groppello
$1,600 \leq X < 2,000$	Sangiovese–Calabrese–Montepulciano–Nebbiolo–Merlot–Teroldego–Lagrein–Nero d'Avola
$2,000 \leq X < 2,400$	Primitivo
$2,400 \leq X < 2,800$	Lambrusco salamino–Croatina–Tamurro
$2,800 \leq X < 3,200$	(none)
$3,200 \leq X < 3,600$	Aglianico–Pinot noir
$3,600 \leq X < 4,000$	Sagrantino

[a]Data as (+)-catechin, mg/kg of grape.

TABLE 7. Percentage of extractable proanthocyanidins (PR) which are localized in the seeds, average data for different cultivars of red grape[a]

Range	Cultivar
$15 \leq X < 20$	Montepulciano–Croatina–Enantio
$20 \leq X < 25$	Nebbiolo–Sagrantino–Syrah
$25 \leq X < 30$	Barbera–Groppello–Nero d'Avola–Teroldego
$30 \leq X < 35$	Negroamaro–Primitivo–Tamurro–Cabernet Sauvignon–Calabrese
$35 \leq X < 40$	Marzemino–Sangiovese–Malavasia nera di Lecce–Lagrein–Merlot
$40 \leq X < 45$	Aglianico–Rebo
$45 \leq X < 50$	Lambrusco salamino–Schiava
$50 \leq X < 55$	(none)
$55 \leq X < 60$	Pinot noir

[a]Data as percentage of the total extractable PR.

TABLE 8. Percentage of extractable catechins and proanthocyanidins reactive to vanillin (VAN), which are localized in the seeds[a]

Range	Cultivar
$35 \leq X < 40$	Tamurro–Croatina–Montepulciano
$40 \leq X < 45$	Nebbiolo–Sagrantino–Enantio
$45 \leq X < 50$	(none)
$50 \leq X < 55$	Sangiovese–Syrah
$55 \leq X < 60$	Nero d'Avola–Malvasia nera di Lecce
$60 \leq X < 65$	Cabernet Sauvignon–Primitivo–Groppello–Calabrese–Teroldego–Negromaro
$65 \leq X < 70$	Schiava–Merlot
$70 \leq X < 75$	Barbera–Aglianico–Lambrusco salamino–Lagrein
$75 \leq X < 80$	Rebo–Marzemino
$80 \leq X < 85$	Pinot noir

[a]Average data for different cultivars of red grape, as precentage of the total extractable VAN.

Ferric reducing antioxidant power (FRAP), total radical-trapping antioxidant parameter (TRAP) and Trolox equivalent antioxidant capacity (TEAC) of alcoholic beverages, teas and coffees[1]

Beverage	FRAP		TRAP		TEAC	
	Value	Rank	Value	Rank	Value	Rank
	(mmol Fe^{2+}/L)		*(mmol Trolox/L)*			
Beer (lager)	2.78	18	NF[2]	22	1.04	18
Chamomile	0.65	20	1.26	19	0.61	19
Coffee (espresso)	129.38	1	66.00	1	36.54	1
Coffee (espresso, decaffeinated)	93.01	4	45.82	4	26.96	4
Coffee (extracted)	96.40	3	59.57	2	30.29	3
Coffee (soluble)	108.56	2	52.37	3	32.48	2
Cognac	2.25	19	1.46	18	1.29	17
Grappa	ND	21	ND	21	0.18	20
Rum	ND	21	ND	21	0.04	21
Tea (black)	10.09	9	4.87	9	3.60	9
Tea (green)	18.00	8	7.63	8	6.01	8
Vinegar (red)	9.50	10	4.80	10	3.12	10
Whiskey	3.45	17	2.31	13	1.68	14
Wine (Aglianico, red)	30.53	6	16.09	5	12.14	5
Wine (Chianti, red)	31.53	5	14.84	6	11.43	6
Wine (Sauvignon, red)	23.90	7	11.73	7	8.95	7
Wine (Villa Torre, rosé)	8.33	11	2.24	14	2.42	11
Wine (Tamerici, rosé)	7.22	12	3.20	11	2.18	12
Wine (Bardolino, rosé)	4.66	14	1.98	16	1.52	16
Wine (Vernaccia, white)	5.04	13	2.32	12	1.94	13
Wine (Pinot, white)	3.72	16	2.10	15	1.68	14
Wine (Greco di Tufo, white)	3.83	15	1.86	17	1.61	15

[1] Values are means, $n = 2$.
[2] NF, not found; ND, not detectable.

Vasodilator effect of procyanidins

TABLE 2. EC_{50} values of the most active peaks

Peak Number	Compound	EC_{50} (C.I.)[a]
E1	Tetramer	2.59 (2.49–2.69)
E3	Trimer-G	1.55 (1.28–1.89)
E4	Tetramer	2.25 (2.14–2.37)
F3	Tetramer	1.54 (1.27–1.87)
F4	Dimer-G	1.25 (0.90–1.73)
F6	Trimer	1.17 (0.96–1.43)
G4	Tetramer-G	0.93 (0.83–1.04)
G5	Tetramer-G	0.57 (0.49–0.67)
G6	Trimer-G	1.00 (0.92–1.09)
G7	Pentamer	1.05 (0.85–1.29)

Adapted from Fitzpatrick *et al.*[6] with permission from the American Chemical Society.

[a]Mean concentration of peak material (μg catechin equivalents/ml) required to produce 50% relaxation (C.I., 95% confidence interval).

sion from the American Chemical Society.)

Vasodilator effect of procyanidins

TABLE 1. Relaxation data and mass spectrometry information on Toyopearl fractions and HPLC peaks

Toyopearl Fraction	HPLC Peak	EDR Threshold[a] (μg/ml)	ES-ITMS Peak Compounds
Fraction A	—	—	gallic acid; other phenolic acids
Fraction B	—	—	flavanol monomers (catechin, epicatechin)
Fraction C	—	>4	monomer-Gal. (ECG); dimers
Fraction D	D 1	—	trimer
	D 3	>4	trimer
	D 4	—	trimer
	D 6	2–3	dimer-Gal.
Fraction E	E 1	1–2	tetramer
	E 2	—	trimer
	E 3	1	trimer-Gal.
	E 4	1	tetramer
Fraction F	F 3	1–2	tetramer
	F 4	1	dimer-Gal.
	F 5	1–2	tetramer
	F 7	0.5–1	trimer
Fraction G	G 2	1–2	pentamer
	G 3	2–4	tetramer
	G 4	<0.5	tetramer-Gal.
	G 5	<0.5	tetramer-Gal.
	G 6	<0.5	trimer-Gal.
	G 7	<0.5	pentamer

Modified from Fitzpatrick *et al.*[6] with permission from the American Chemical Society.
[a]Amount of fraction or peak material required to produce 15% relaxation.

Vasodilator effect of procyanidins

TABLE 2. EC_{50} values of the most active peaks

Peak Number	Compound	EC_{50} (C.I.)[a]
E1	Tetramer	2.59 (2.49–2.69)
E3	Trimer-G	1.55 (1.28–1.89)
E4	Tetramer	2.25 (2.14–2.37)
F3	Tetramer	1.54 (1.27–1.87)
F4	Dimer-G	1.25 (0.90–1.73)
F6	Trimer	1.17 (0.96–1.43)
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G5	Tetramer-G	0.57 (0.49–0.67)
G6	Trimer-G	1.00 (0.92–1.09)
G7	Pentamer	1.05 (0.85–1.29)

Adapted from Fitzpatrick *et al.*[6] with permission from the American Chemical Society.

[a]Mean concentration of peak material (μg catechin equivalents/ml) required to produce 50% relaxation (C.I., 95% confidence interval).

Wine as essential food in nutrition?

- The studies reported in this course suggest that alcohol can act as immunomodulator in inflammation and coagulation, counteracting the development of the atherosclerotic disease.

- The concordance of such results from the epidemiological studies, laboratory testing and clinical studies have encouraged the experts, nutritionists and clinicians to introduce small amounts of alcohol in the diet of healthy adult.

- This change, potentially important from the scientific and health point of view may, however, hide damaging misconceptions and feed dangerous distortions as a result of enthusiasm and interested speculations.

Wine as essential food in nutrition?

- Current researches indicate that wine exerts its positive effects only if correctly added (for quantity, methods and timing) in a balanced diet.

- Concerning the various and numerous phenolic and polyphenolic substances present in alcoholic drinks, we should know more about the bioavailability, absorption and the mechanisms of action in vivo, to elucidate the benefits of each specific component.

- The benefits that wine can perform as food are not simply related to the presence of specific substances, but also to their activities in the context of a specific feed formulation and an appropriate diet.

- Finally, the possible benefits expected from the correct use of wine, should not confuse the consciousness about the serious risks of abuse or uncontrolled intake of wine for human health.