CHEMISTRY IN WINEMAKING

Wine has importance in many social and religious contexts in our society today, as it has for thousands of years. The basic fermentation process whereby alcohol is produced from the sugar in grapes is very simple, but its chemistry is still not completely understood. As this knowledge increases, winemakers are being helped to improve the quality of their wine.

Grape juice consists of 79% water and 20% carbohydrates, 1% organic acids and trace amounts of organic acids, phenolics, vitamins, minerals and nitrogenous compounds. The sugars, organic acids and phenolics give the juice its flavour, while the vitamins, minerals and nitrogenous compounds are, in many cases, essential to yeast growth and fermentation. Wine has a similar composition, but has much lower levels of sugar (none in dry wines), 8 - 13% alcohol and a greater range of minor components. Commercial wine manufacture consists of five basic steps.

Step 1 - Harvesting
This is the most critical stage of the process. The grapes must be harvested when the sugar, acid, phenol and aroma compounds are optimised for the style of wine desired.

Step 2 - Crushing and destemming
The grapes are removed from the stems and gently crushed to break the skins. Sulfur dioxide is added to the grapes at this stage to prevent oxidation and inhibit microbial activity. Enzymes may also be added to break down the cell walls and aid the release of juice.

Step 3 - Pressing
The juice extraction process depends on the type of wines to be used, but always involves squeezing the berries. After pressing the juice is allowed to stand to separate the solids. If necessary the juice may be clarified by filtration or centrifugation.

Step 4 - Fermentation
The juice is innoculated with live yeast, which then carries out the fermentation reaction:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

This reaction occurs through many intermediary biochemical steps. The process is carried out under a blanket of carbon dioxide as in the presence of oxygen the phenols are oxidised and the sugar and ethanol are converted to carbon dioxide and water.

Step 5 - Purification
Unwanted solids, salts and microorganisms are removed through a variety of physical processes, then the wine is bottled and sold.

The laboratory is involved throughout the process, and particularly at the harvesting, fermentation and purification steps, which need to be monitored closely.

Wine making could present a significant environmental problem as a large volume of waste with a high BOD (biological oxygen demand) is produced, but this is usually composted or otherwise disposed of on site, so the environmental hazard is minimised.

INTRODUCTION

Wine is of great importance in our society today, and has been so for thousands of years. Grapes have been cultivated for wine production in the Near East since 4000BC, and in Egypt since 2500BC. They were spread from the Black Sea to Spain by the Greek Empire, into Germany by the Romans and to the New World by Columbus. Wine has had religious significance as both an offering and a sacrament since Biblical times, and this has helped its development. Today an enormous variety of wines are available, made from more than 5000 varieties of a single species of grape: *Vitis vinifera*. In the production of all these wines, chemistry is important, and as some of the complexities of wine chemistry have begun to be understood chemists have been able to contribute greatly to the improvement of wine quality.

THE COMPOSITION OF GRAPES

The composition of grapes is of great importance in determining the quality of the wine produced. Many compounds are carried over from the grape juice into the wine, and other compounds undergo reactions to form the compounds distinctive to wine. It was once common to ameliorate the wine produced with a variety of chemical treatments, but now this is frowned upon. In this new climate, using high quality grapes is essential to producing high quality wines, and grape composition is more important that ever.

In general, grapes consist of clear juice (80%), skins (8%), seeds (4.5%), pulp (4.5%) and stems (3%). The skins, seeds, pulp and stems are collectively known as 'pomace'.

Another way of looking at grape composition is in terms of chemical components, and these are summarised in **Table 1**. However, it is important to realise that the quantity of a given ingredient is not directly related to its importance. For example, vitamins (which are present in such low concentrations that they do not appear in the table) are very important for yeast growth and hence for fermentation. The major constituents of grapes are examined in more detail below.

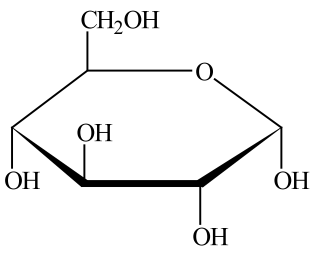
Carbohydrates

Carbohydrates are molecules of the general formula $C_x(H_2O)_x$, and sugars are a sub-group of carbohydrates. They consist of up to 10 'monosaccharides' - carbohydrates that cannot be broken down into two new carbohydrates. Usually these do not consist of more than seven carbons. Sugars are sweet-tasting, water soluble and good energy sources. Higher carbohydrates generally have structural functions.

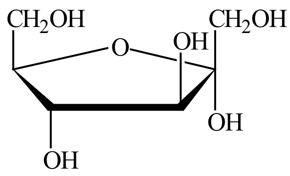
The most important sugars in grape juice are the two six-carbon sugars glucose and fructose. These are the sugars that make the juice sweet and are fermented to alcohol by the yeast. In addition, small quantites of pentoses (five carbon sugars) and pectins (galacturonic acid polymers) are found. The pectins have no great importance in the juice itself, but if they are not broken down they can create haziness in the wine.

Table 1 - Composition of grape juice and wine (mean values in %w/w unless otherwise stated)

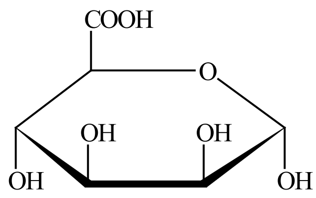
	Juice	Dry Wines
Water	79	85
Carbohydrates (total)	21	0.2
Fructose	11	0.07
Glucose	10	0.06
Pectin	0.06	0.2 (as galacturonic acid)
Pentoses	0.1	0.1
Alcohols		
Ethanol	trace	12.5 (v/v)
Glycerol	0	0.6 - 1.0
Higher alcohols	0	0.02 - 0.04
Methanol	0	0.01
Aldehyde	trace	0.01
Organic acids	0.8	0.7
Acetic	0.01	0.03 - 0.07
Amino acids (total)	0.04	0.1 - 0.25
Citric	0.02	0.02
Lactic	0	0.03 - 0.5
Malic	0.1 - 0.8	0.0 - 0.6
Succinic	0	0.1
Sulphurous	0	0.02
Tartaric	0.6 - 1.2	0.5 - 1.0
Phenolics		100 - 2500 mg L^{-1}
Simple		6 - 150 mg L^{-1}
Hydrolysable tannins		T (red and chardonnay)
Condensed tannins		50 - 800 mg L^{-1}
Anthocyanins		0 - 1000 mg L^{-1}
Nitrogenous compounds	0.12	0.03
Amino	0.07	0.1
Ammonium	0.006	0.03
Protein	0.005	0.01
Resdiual	0.015	0.01
Minerals (ash)	0.4	0.3
Calcium	0.015	0.004 - 0.01
Chloride	0.01	0.005 - 0.02
Magnesium	0.015	0.004 - 0.012
Phosphate	0.03	0.0025 - 0.085
Potassium	0.2	0.06 - 0.12
Sodium	trace	0.004
Sulphate	0.02	0.07 - 0.3



glucose



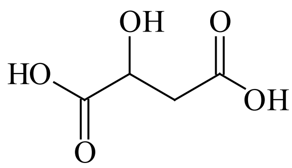
fructose



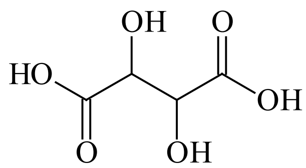
galacturonic acid

Organic acids

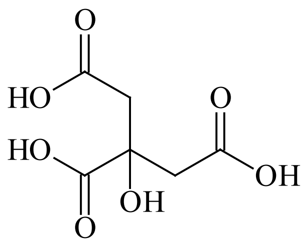
Three main organic acids occur in grapes: malic, tartaric and citric acid. Of these, tartaric acid is rare in fruits but the others occur widely. A fourth acid, succinic acid, is formed from yeast metabolism and so is found in wine but not in grapes. The acids give the juice its acidity, and act as an effective buffer to maintain the pH at around 3.2 - 3.3. They are also important contributors to the flavour balance of the juice and wine, providing the sharp acidity.



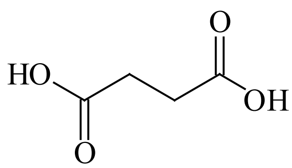
malic acid



tartaric acid



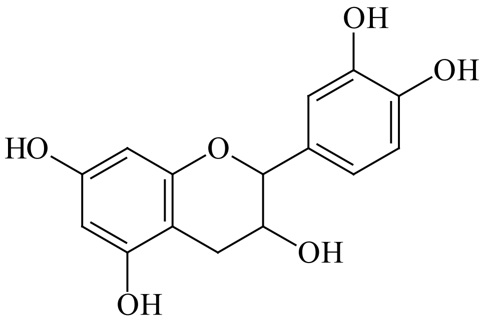
citric acid



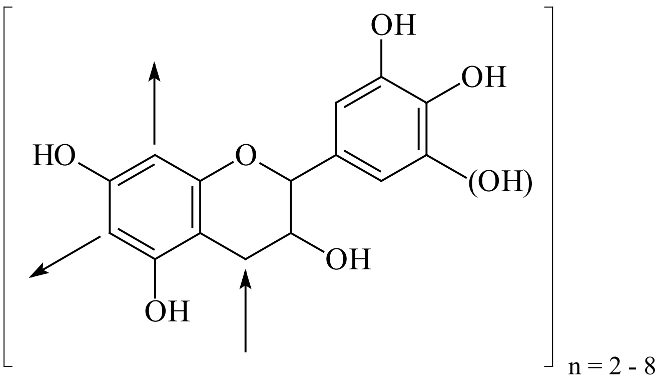
succinic acid

Phenolics

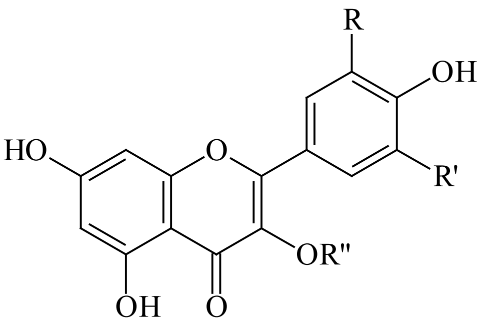
The term 'phenolics' refers to a large group of compounds containing at least one phenol group (i.e. containing at least one hydroxylated benzene ring). These compounds contribute to the astringency / bitterness of the grapes and wine and are responsible for most of the colour. There are six main classes of phenolics found in grapes: catechins, procyanidins, anthocyanins, flavonols, hydroxycinnamates and hydroxybenzoates. The difference between red and white wines is due to the different types of phenolics in the two beverages. The simple phenolics - the hydroxycinnamates and hydroxybenzoates - occur in the flesh of the berry and so occur in both red and white wines. The other more complex phenolics, known collectively as flavanoids, occur in the skin, seeds and stems and so occur mostly in red wines. The procyanidins are also known as condensed (or non-hydrolysable) tannins, and it is these that give wine most of its astringency. A further group of tannins, the hydrolysable tannins, are found in wine that has spent time in oak barrels. These tannins are also astringent, and are complex esters of glucose and gallic acid. Anthocyanins are the commonest source of colour not only in grapes but in all flowering plants. Their colour depends on the number of hydroxyl groups on the molecule and can range from orange through red to purple.



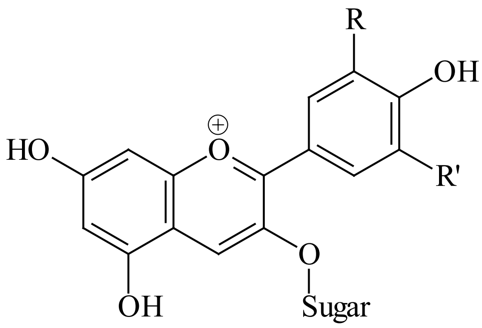
catechins



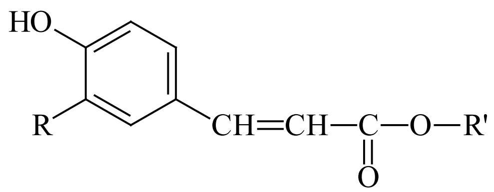
procyanidin polymers



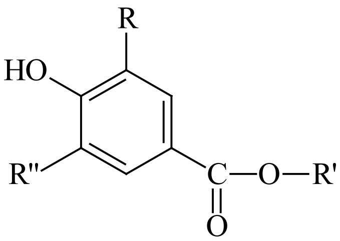
flavonols



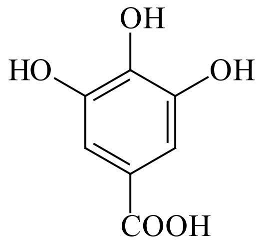
anthocyanins



hydroxycinnamates



hydroxybenzoates



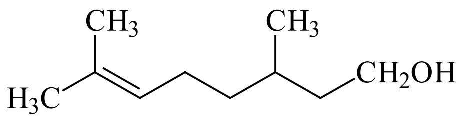
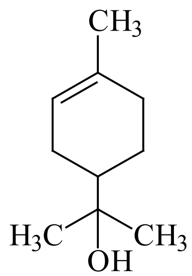
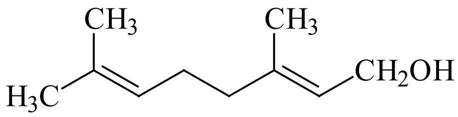
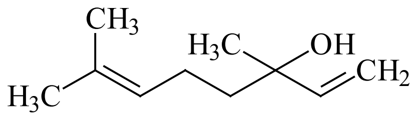
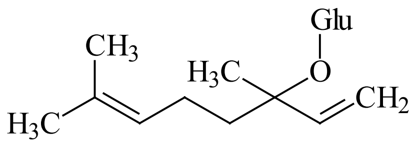
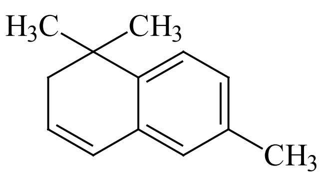
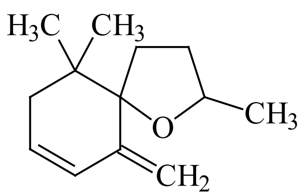
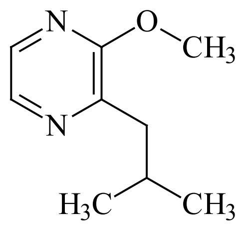
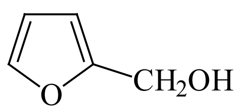
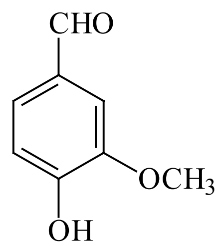
gallic acid

Aroma compounds

The basic flavour of a wine is formed from the balance of sugars, acids, phenolics, and ethanol, but the *character* of the wine is provided by the volatile aroma compounds. Over 1000 of these volatiles have been identified in wines from around the world, all present at low, almost trace, levels. The most important volatiles in the grape are the monoterpenes, present even in aromatic varieties such as the Riesling at concentrations no higher than 4mg kg^{-1}. These give a range of odours ranging from floral / fruity to resinous / solvent effects. Examples of the most important monoterpenes are shown in **Table 2**. The monoterpenes exist in both the free volatile form and as bound glycosides. The glycosides are not volatile and so do not contribute to the aroma. They are slowly hydrolysed in the acid condidtions of the wine and contribute volatiles as the wine ages. During processing and aging of the wine these monoterpenes are also converted to other more complex volatiles such as TDN and vitispirane.

The terpenes are not the only contributors to the grape aroma. For example, the well known

Table 2 - Some aroma compounds found in wine

Class	Examples
Monoterpenes	 Citronell  α-Terpineol  Geraniol  Linalool
Glycosides	 Linaloyl glucoside
Other volatiles	 1,1,6-Trimethyl-1,2-dihydro napthalene ("TDN")  Vitispirane  Isobutyl methoxypyrazine  Furfuryl alcohol  Vanillin

'Sauvignon' aroma of Carbernet Sauvignon and Sauvignon Blanc is due to methoxypyrazines, and the 'foxy' aroma of North American native grapes (i.e. grapes of a different species from the typical *vinifera* species) is due to the ester methyl anthranilate.

The yeast also contributes volatiles both from its own metabolism and by modification of compounds in the grape juice. This results in the formation of the fusel oils (alcohols) such as 3-methyl butanol and 2,3-butandiol. Volatile phenols such as vinyl phenols are also produced by decarboxylation of the hydroxycinnamates.

Other contributors to the wine aroma are esters such as ethyl acetate and hexyl acetate (formed from interactions between the alcohols and acetic acid), furans such as furfuryl alcohol (formed from the decomposition of sugars) and vanillin (extracted from the oak barrels).

Nitrogenous compounds
There are many sources of nitrogen in grapes (e.g. DNA, enzymes, inorganic nitrogen etc.) but the most common (and most significant) of these are amino acids. As free acids they are important building blocks for yeast during fermentation[1]. In addition, many enzymes retain their catalytic activity in grape juice. Two groups of enzymes of significance are the pectinases ("pectolytic" enzymes which hydrolyse pectins, preventing them from forming a haze in the wine) and the phenol oxidases (which cause browning reactions to occur in the juice unless they are inhibited by dissolved SO_2).

Vitamins
These are present in very low levels - too low to be included in **Table 1**. However, although grapes are not a very good source of vitamin C they do contain some vitamins in large enough concentrations to be useful in human nutrition. Some of the main vitamins found in grapes are listed in **Table 3**.

Table 3 - Vitamins present in grapes

Vitamin	**Quantity / g L^{-1}**
Inositol	500 000
Nicotinamide	3 260
Pantothenate	820
Pyridoxine	420
Riboflavin	21
Cobalamine (B_{12})	0.05

Minerals
The minerals in grapes are those found in the soil in which the grape vine was growing, so mineral content varies greatly from vineyard to vineyard. In general, minerals make up 0.4% of the weight of the grapes. The most important minerals are magnesium and potassium, which are important in fermentation, and phosphate, which is necessary for yeast growth.

[1]Note that yeasts have limited ability to hydrolyse proteins, so whole proteins are of limited use to them.

THE WINE MANUFACTURING PROCESS

Step 1 - Harvesting

As the grapes ripen the concentration of sugars and aroma compounds rises and the concentration of acids falls. The aim at harvest is to pick the grapes at their optimum composition. This depends on the type of wine to be produced. For example, sparkling wine requires a higher acidity than still table wine. The development of the grapes is followed by taking samples of the grapes at regular intervals from a few weeks before the expected optimum levels will be reached. The samples are analysed for pH (using a pH meter), acid (by titration with sodium hydroxide), sugar (by refractive index or chemical reduction of copper salts) and flavour compounds (by tasting). When optimum levels are reached, the grapes are harvested.

Step 2 - Crushing and destemming

Sulphur dioxide (5 - 10% solution of metabisulphite) is usually added to the grape bunches as they are fed into the crusher/destemmer. The stems are removed as the bunches pass through a perforated rotating cylinder in which the grapes fall through the perforations while the stems are separated out by beathers. The berries are then passed through rollers and crushed. The SO_2 inhibits the growth of wild microorgansisms and prevents oxidative browning of the juice. Molecular SO_2 is the active biocide, but in solution this is in equilibrium with inactive HSO_3^-. At wine pH only 2 - 8%[2] of the SO_2 exists in the molecular form, but this is usually sufficient to give the required protection. Wherever possible during the manufacturing process the juice is kept under a blanket of CO_2 to exclude air, and if necessary more SO_2 is added to maintain the level of molecular SO_2 at a minimum of 80ppm.

Step 3 - Juice preparation

The free-run juice is separated from the crushed berries, which are pressed by gentle squeezing to obtain a high quality juice. The juice is allowed to settle overnight or is centrifuged to clarify it. If necessary pectolytic enzymes are added to remove haze. Finally, the pulp is then squeezed almost dry. This final juice is of low quality and is used for cask wine or fermented for distillation into alcohol for sherry or port production.

Step 4 - Fermentation

Fermentation is begun by innoculating the juice with the chosen wine yeast. This yeast catalyses a series of reactions that result in the conversion of glucose and fructose to ethanol:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

The driving-force behind this reaction is the release of energy stored in the sugars to make it available to other biological processes. In aerobic conditions, the reaction can proceed further and convert the ethanol to H_2O and CO_2, releasing all of the energy present in the original sugars. This process is undesirable in wine production, so fermentation is usually carried out under a blanket of CO_2 to exclude oxygen and hence maximise alcohol production. The chain of biochemical reactions involved is shown in **Figure 1**. Depending on the conditions,

[2] The exact concentration of SO_2 depends on the wine pH, which in turn depends on the grapes. The legal maximum level is 200ppm, with levels of 80 - 140ppm being typical.

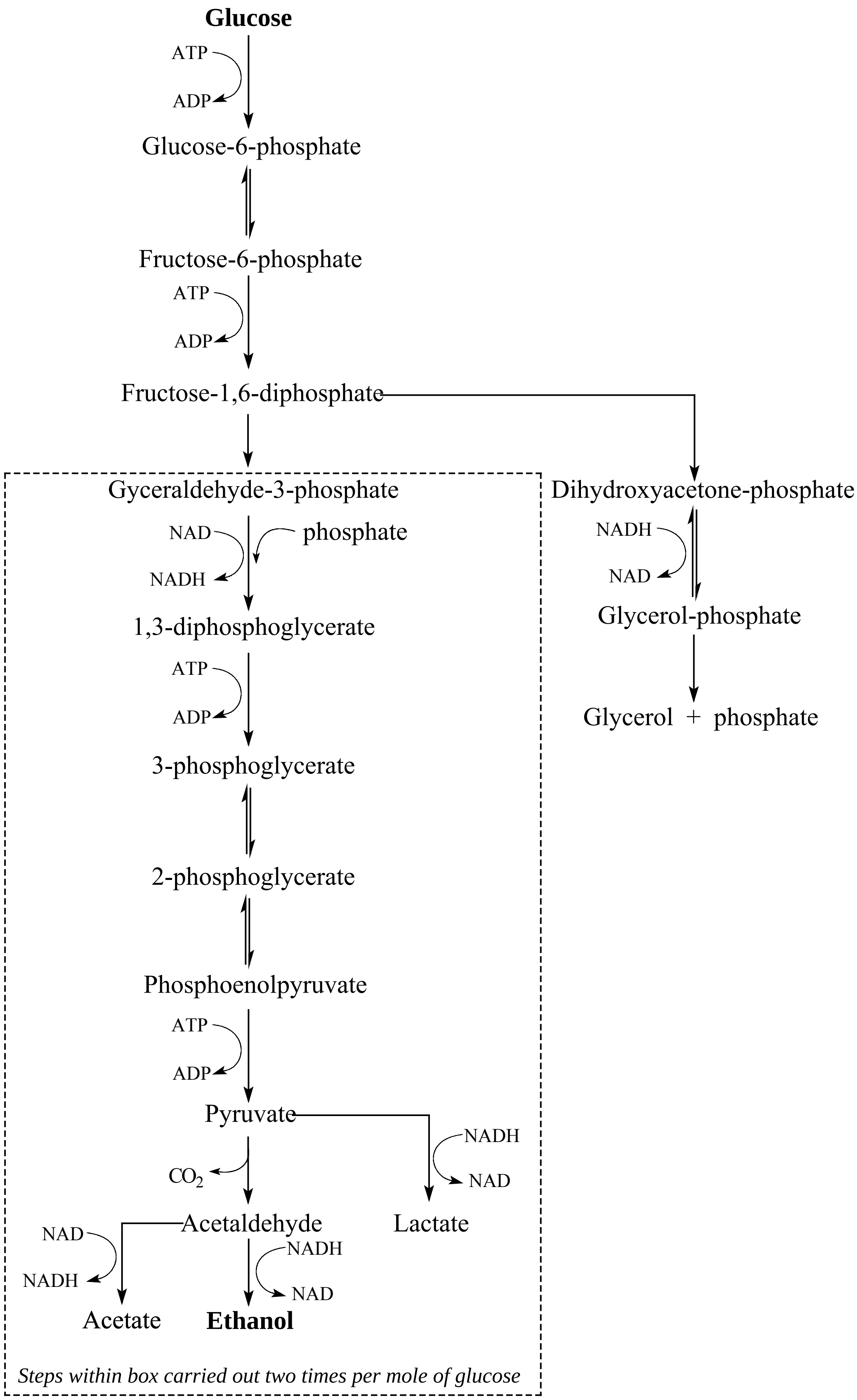


Figure 1 - Glycolysis and alcoholic fermentation

various intermediates in the fermentation process can be converted into other products. The main ones are listed in **Table 4**.

The yield of ethanol is affected by such factors as temperature, extent of agitation, sugar concentration, acidity, strain of yeast and yeast activity. The lower the temperature the higher the alcohol yield due to a more complete fermentation (better sugar utilisation) and less loss of alcohol entrained with CO_2. While it may be important to maximise the yield of ethanol it is equally important that this is never achieved with complete efficiency. The various byproducts of yeast metabolism formed by this inefficiency contribute to wine's distinctive flavour and aroma and prevent it from simply being alcoholic grape juice.

The juice used to be fermented in wax-lined concrete or plastic vats, but now stainless steel is used for all wines except for certain high-quality ones that are fermented in wood. Wooden barrels are the container of choice for chardonnay, sauvignon blanc and pinot noir as the wood is smoked during processing, forming additional flavour compounds (particularly tannins) which are leached into the wine, giving it further complexity.

Step 5 - Purification

In former times, after fermentation was complete, the wine was heavily treated to alter the pH, composition etc. to give it a desirable flavour, appearance etc. Very few such measures are used today, but those that are retained are outlined briefly below.

Proteins and tannins that are suspended in colloidal form in the wine are precipitated out with substances such as gelatin or adsorbed to the surface of substances such as bentonite. This process is called *fining*. The wine is often also clarified in a process called *racking*. This is the drawing off of the wine from the lees (sediment formed). Wine is often also *cold stabilised* (left at 0 to -3°C for 10 - 14 days) to crystallise out any potassium bitartrate

Table 4 - Products of alcoholic fermentations

Product	Theoretical %w/w	Industrial fermentation %w/w	Wine yeast %w/w
Ethanol	51.1	48.4	47.86
CO_2	48.9	46.5	47.02
Acetaldehyde	—	0.08	0.01
Acetic acid	—	0.25	0.35
Glycerol	—	3.6	2.99
Lactic acid	—	0.2	0.20
Succinic acid	—	0.7	0.045
Higher alcohols	—	0.33	0.1
Yeast mass (dry weight)	—	1.2	0.55

(KHTa). These treatments are only usually necessary in white wine as red wine fines and clarifies itself by forming deposits of proteins, tannins and tartrates during the ageing process, although sometimes proteinaceous fining agents are added to modify tannin levels and structures. The wine is continually racked off this precipitate, such that by the end of the ageing process all it needs is simple filtration before bottling and sale.

THE ROLE OF THE LABORATORY

The analytical laboratory is involved throughout the wine making process, from harvesting to bottling. Tests are also carried out from time to time by the state to ensure that illegal 'improvements' (such as the addition of glycerol) have not been made by the wine manufacturer. A selection of the standard tests used is given below.

- To determine when to harvest the wine, sugars and organic acids must be determined. Sugars are determined by measuring the specific gravity or refractive index of a juice sample. Organic acids are determined by titrating 10 mL of a diluted sample with standard 0.1 mol L^{-1} sodium hydroxide to the phenolphthalein endpoint. The result from this test is usually expressed as g L^{-1} equivalent of tartaric acid as this is the most common acid present in grape juice.

- To determine when fermentation is nearly finished, specific gravity is monitored. The juice initially has a specific gravity greater than one, due mainly to dissolved sugars. When the specific gravity falls to 1.000, the wine is nearly ready. This can be simply monitored by testing specific gravity, but some manufacturers also use "Clinitest" tablets (as used by diabetics) or the Fehling reaction for more precise monitoring as the specific gravity approaches 1.000. The Fehling reaction is as follows:

$$RCHO(aq) + 2Cu^{2+}(aq) + 4OH^{-}(aq) \rightarrow RCOOH(aq) + Cu_2O(s) + 2H_2O(l)$$

  The colour of the resulting solution indicates the amount of sugar left. A large amount of sugar results in complete loss of the blue copper (II) ions leaving the red copper (I) oxide. Less sugar and some blue copper (II) ions remain and less red copper (I) oxide is formed. No sugar and the solution remains blue.

- The amount of fining agent necessary is determined by taking 100 mL samples of wine, treating them with a suspension of the fining agent, and leaving them overnight. Very small amounts of fining agent are necessary, with 15 g of gelatin per 100 L of wine usually being sufficient. White wines are usually fined with bentonite, with the completeness of the fining tested by heating or with phosphomolybdic acid.

ENVIRONMENTAL IMPLICATIONS

Wine effluents have a high BOD and a low pH, as well as containing significant amounts of sulphur and phosphates. For this reason the effluent must be carefully treated to avoid contamination of local waterways. Disposal needs are also highly seasonal, with both BOD and volume peaking during vintage.

Dry waste
Winery dry waste is dealt with in a variety of ways. Some wineries are located in municipalities and are able to discharge all of their wastes into standard commercial waste disposal systems, but wineries in country locations need to use special procedures. For some time such wineries have spread grape skins and pips in vineyards to decompose over a large area, and some wineries are now also composting this waste to further protect the environment and integrate their operation. However other dry wastes such as filter cake and diatomaceous earth are still always disposed of in landfills.

Liquid waste
Commonly the liquid is left to settle (often in an aerated pond) to separate out solids and reduce its BOD. The solids are then either returned to the vineyard or landfilled and the liquid used for irrigation in pastures or amongst tree plantations. Often the liquid must be diluted or neutralised to an acceptable pH, as winery wastes are generally acidic (although the caustic solutions used for cleaning tanks occasionally cause the effluent to be basic).

Aside from winemaking itself, bottling can also create effluent problems as cleaning bottles for reuse requires washing with caustic soda ($NaOH$). However, wine is currently usually bottled in new bottles, so this is only rarely a problem, and when bottles are recycled they are usually recycled by a commercial recycler rather than the winery. Thus bottling no longer creates an effluent problem for winemakers.

Written by Heather Wansbrough with assistance from Dr. Robert Sherlock and Dr. Maurice Barnes (Lincoln University) and Malcolm Reeves (Cross Roads Winery Ltd.) and with reference to:

- Moletta, Dr. René and Baudel, Julienne; *Congrès international sur le traitement des effluents vinicoles*; CEMAGREF; 1994

- Linskens, H. F. and Jackson, J. F.; *Wine Analysis*; Springer-Verlag; 1988

- Jackson, David and Schuster, Danny; *The Production of Grapes and Wine in Cool Climates*; Butterworths of New Zealand; 1987

and the article in the previous edition of *CPNZ* by Dr. D.E.G. Sheat (Ruakura Agricultural Research Centre) and A.R. Eames (Hamilton Boys' High School).