The effects of alternating electric fields on wine

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Statement of Originality

I declare that this submission is my own work, and to the best of my knowledge, it contains no material previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at any educational institution, except where acknowledgement is made.

Signed:

Date:

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Abstract

Maturation of wine, also called ageing, is an important process in making premium wine. During this process, many compounds change in concentration, and these changes are responsible for colour and flavour changes that contribute to the complexity of matured wine. However, ageing is an expensive process due to the time taken, frequently years, the cost of which cannot be recovered until sale. Alternating electrical fields have purportedly been shown to accelerate ageing of wine as judged by informal sensory trials, and by limited results in the scientific literature.

This research aimed to quantity the changes due to electric fields in terms of sensory and chemical science. Wine ageing machines (WAM) equipped with alternating electric fields were used to treat red wines (Cabernet Merlot, Pinot Noir and Merlot Cabernet Sauvignon) and white wine (Sauvignon Blanc). There were three versions of WAM: a flow-through WAM from the University of Waikato, a flow-through WAM2 (a modified version of the original), and a static WAM. In the first two, the wine flows through glass tubing between the charged plates 1 cm apart. In the static WAM, tubes containing wine are suspended between vertical plates. In the present study, electric fields were adjusted with different frequencies and strengths. The wines were physicochemically analysed by ultraviolet spectrometry, liquid chromatography mass spectrometry (LC-MS) and liquid chromatography mass spectrometry/ mass spectrometry (LC-MS/MS). Further, sensory tests were conducted between control wines and WAM-treated wines. Only flow-through WAM2 treated Sauvignon Blanc appeared to show an absorbance difference in the wavelength range of 230 to 350 nm, but the difference was finally interpreted as artifactual arising from electronic noise in the spectrophotometer. Further, other WAM-treated wines did not show any sensory or colour differences compared with control wines that could not be attributed to noise. In LC-MS (/MS) studies, several compounds were shown to vary with treatment, notably 2-furoic acid in white wine. However, the differences observed were inconsistent and might have been due to statistical variation that can arise in 1 in 20 events by chance alone. It was concluded that these WAMs had no effect on wine ageing in the manner they were applied here.

Chapter 1

Introduction

1.1 Alcoholic drinks

According to McGovern et al. (2004), purposely fermented beverages existed as least as early as 10,000 Before Present and the archaeological record is rich in evidence for alcoholic fermentation from very many cultures throughout the world. The carbohydrate source was a varied historically with time and culture as it is today, when alcoholic drinks are routinely fermented from grains, fruits including grape, vegetables, palms and honey. The last, honey wine, is proposed to be the "ancestor of all fermented drinks" (Maguelonne, 1993). This seems entirely likely because fermentation would require only the addition of water in a container of some description and colonisation of the mixture by wild microorganisms.

Commercial fermentation in the modern world is dominated by beers from grains, principally barley and rice, wine from grapes of the genus *Vitis*. These fermented beverages are also purpose fermented as a base for distillation that ultimately generates a range of spirits like vodka (schnapps), whisky, gin and brandy. Unlike wild fermentations of honey for example, each of the modern fermentations is initiated by inoculating the base carbohydrate/water mixture with a known yeast culture that results in more predictable quality outcomes.

It is well known that many alcoholic drinks, especially those with a high alcoholic content, improve in flavour by storage for months, years, and even decades in the case of whiskies. In the case of beers with alcoholic contents between 3 and 6 % (v/v)[1](rarely higher), this maturation phenomenon is called 'conditioning'. For wine and whisky it is called 'maturation' or 'ageing'.

Reverting to the overall term 'ageing', ageing always carries a cost, either to the seller or to the buyer. When the producer/seller sells the liquor immediately and the buyer/ultimate consumer holds the liquor for the duration of ageing, the liquor is effectively 'dead' money during the ageing period.

[1] Percent alcohol v/v is assumed throughout this thesis.

Conversely, if the producer/seller holds the liquor unsold during ageing, there is a major cost because cost of production and storage cannot be recovered for years sometimes. Thus there is a commercial incentive to decrease ageing time. This is the subject of this thesis. First, however, there is a description of the major liquor classes, and fermentation, but eventually focusing on wine and its ageing.

1.1.1 Beer

Beer comes from the Latin word *bibere*, meaning "to drink" (Baiano, Conte, & Alessandro Del Nobile, 2012). About 6,000 years ago, it is believed that the ancient Sumerians noticed that an alcoholic pulp resulted from the fermentation of wet bread or grain. This happened by chance in that they had no known of yeast culture. Sumerians created the oldest recipe for beer described "Hymn to Ninkasi"(Baiano et al., 2012). In the recipe, dough and honey were used for fermentation.

In the modern world, beer is defined as the product of an alcoholic fermentation of sugars derived from malted cereal to which hops have frequently been added. Beer is a relatively low alcohol content beverage, ranging from 0.5 to 12% but usually between 3 and 6%. Different countries have specific regulations on alcohol content of beer (Baiano et al., 2012; Xu, 2008). Traditional cereal sources are barley, wheat, corn and rice. Other ingredients such as hops, water, sugar, yeast and bacteria are used in the production (Xu, 2008). Broadly, two types of beers are sold worldwide: lager and ale (Kodama, Kielland-Brandt, & Hansen, 2006). The types of beer is reflected by the types of yeasts used for the fermentation (Kodama et al., 2006). Lager beer is the beer produced from bottom fermentation by the lager yeast *Saccharomyces carlsbergensis*. Ale beer is the beer produced from top fermentation conducted by ale yeast *S. cerevisiae* (Bekatorou, Psarianos, & Koutinas, 2006). Lager beers are pilseners, bocks, American malt liquors etc. and ale beers are porters, stouts and wheat beer etc.(Bekatorou et al., 2006).

Beer has considerable nutritional value in that it contains non-fermentable and/or non-fermented carbohydrate, as well as B vitamins such as thiamine, riboflavin, niacin, B_6 and B_{12}, and folic acid (Bamforth, 2002; Kaplan & Palmer, 2000). The concentration of niacin in beer is very high at about 3 to 8 mgL^{-1}(Bamforth, 2002). Beer also contains significant amount of elements such as magnesium, selenium, potassium, phosphorus (Bamforth, 2002; Kaplan & Palmer, 2000). All of

these nutritional components were derived from the original cereal.

1.1.2 Wine

Wine is produced from alcoholic fermentation of fruit of or other vegetative matter (Margalit, 2004). According to that author, there are three varieties of wine: grape wine, fruit wine and agricultural wine. Grape wine is produced from fermentation of juice of ripe grapes of the genus *Vitis*. Fruit wine is produced from fermentation of ripe fruit. Agricultural wine is produced from fermentation of vegetative agricultural product other than grape, fruit, molasses and grains (Margalit, 2004). The minimum alcohol content of grape wine and fruit wine is not less than 7%. Grape wine, the alcohol concentration of which is between 9 to 14% (v/v) is usually called table wine (Jackson, 2008; Margalit, 2004). Grape wine can be subdivided into still table wine and sparkling table wine based on carbon dioxide content. Still table wine can be divided into white, red and rose groups based on the colour (Jackson, 2008). Fortified wines are usually red wines to which pure ethanol is added, ultimately yielding products where the alcohol content is between 17 and 22% (Jackson, 2008). Fortified wines include sherry, port and similar wine.

Most wines are consumed in a food-related setting, proving a range of flavours, acidity and sweetness to balance the food involved. In general terms, white wines accompany lighter food styles like fish, whereas red wines accompany heavier styles like steaks. Fortified wines are usually consumed at the end of meal.

In common with beer, wine has some nutritional features. Grape wine at least is a good source of phenolic and polyphenolic compounds: nonflavonoids, flavanols, anthocyanins and flavan-3-ol (Bamforth, 2002; Jackson, 2008; Margalit, 2004). These are derived from skin and pulp of the grapes.

Composition of wine

There are five major groups of chemicals in wine: sugars, acids, ketones/aldehydes, esters and phenols.

Sugar in wine usually comes only from grapes. The sugars in grape are glucose, fructose, sucrose, rhamnose, arabinose, xylose, gum and pectin. The monosaccharides glucose and fructose are the principle sugars in grape juice (Jackson, 2008; Margalit, 2004). Their concentration ratios in grapes

are similar, but their absolute concentrations vary with grape variety, physiological status and maturation of grapes (Jackson, 2008). Sucrose is rarely found in the *Vitis* grape. As a result of fermentation, the concentrations of glucose, fructose and sucrose obviously decrease during fermentation. However, the concentrations of rhamnose, arabinose, xylose increase during wine maturation in oak barrels due to their liberation from complexes such as glycosides present in toasted wood (Jackson, 2008). Gum and pectin are polysaccharides derived from the cell walls in grapes. They are relatively less soluble in alcohol compared with other components in the fermentation mixture, and thus precipitate during fermentation (Jackson, 2008; Margalit, 2004).

Alcohols are the major residual product of fermentation or are the products of the reactions that are not directly included in the fermentation pathway (Margalit, 2004). Thus, ethanol is the major product from alcoholic fermentation, whereas methanol is the product of hydrolysis of pectin by methylesterase enzymes that naturally occur in ripe grapes. Higher alcohols in wine are formed from amino acids in grapes or from the metabolic activities of spoilage yeasts and bacteria (Jackson, 2008; Margalit, 2004). Examples of higher alcohols in wine are 3-methylthio-1-propanol and propanol and isopropanol. 3-Methylthio-1-propanol contributes a sweet aroma to wine (Margalit, 2004). Polyols such as 2,3-butanediol and glycerol are also found in the wine. Glycerol contributes to the wine body and mouthfeel due to its sweet and viscous character (Jackson, 2008; Rankine & Bridson, 1971).

Aldehydes and ketones are formed during alcoholic fermentation. Acetaldehyde, acetoin and diacetyl are the major aldehydes and ketones found in wine. Acetaldehyde forms during fermentation, and it is dominantly reduced to ethanol in the reductive environment of anaerobic fermentation, but reduction is not usually complete. Later, when wine is exposed to oxygen, ethanol is oxidised to acetaldehyde. Acetoin and diacetyl, which both contribute buttery notes in wines, are also products of fermentation by yeasts.

Acid contributes a tartness to wine. The major acids in wine are tartaric, citric, malic, succinic, lactic and acetic (Margalit, 2004). Tartaric, citric and malic acids are derived from grapes, while succinic, lactic and acetic acids are formed during fermentation (Jackson, 2008; Margalit, 2004).

Esters in wine result from the dehydration reaction between alcohol and acid, and in the case of

wine the esters come from the original grapes, or are generated during fermentation and ageing (Margalit, 2004). Esters are important for the aroma of wine on account of their intrinsic volatility. The most important ester in wine is ethyl acetate. It is responsible for fruity aroma at low concentration but nail varnish aroma at high concentration (Sumby, Grbin, & Jiranek, 2010). Other major esters in wine include isoamyl acetate (banana aroma), ethyl oactanoate (sweet, fruity, beer aroma), and ethyl hexanoate (strawberry, green apple aroma) (Margalit, 2004; Sumby et al., 2010).

Two groups of phenols exist in grape and wine: flavonoids and non-flavonoids. Major flavonoids in wine include flavonols, anthocyanidins and flavan-3-ols (Kennedy, 2008; Waterhouse, 2002). Flavonoids have carbon skeleton of C_6-C_3-C_6 (Figure 1) (Margalit, 2004). Flavonoids are derived primarily from skin and seeds of the fruit. Flavonols and anthocyanidins occur in the cellular vacuoles of the grape skin. Derivatives of anthocyanidins (usually bound to glucose) are called anthocyanins, and these are the red and blue pigments responsible for the colour of red wine. Flavan-3-ols are found in the stems and seeds of *Vitis*. Flavan-3-ols include catechin, epicatechin, gallocatechin, procyanidins and condensed tannins (Jackson, 2008). The tannins consist primarily of catechin, epicatechin and gallateepicatechin subunits. Most phenols are bound to polysaccharides in the cell wall (Tian, Nakamura, & Kayahara, 2004). Flavonoids constitute more than 85% of the phenol content of red wine but less than 20% of white wine. They characterise red wines more than they do white wines (Jackson, 2008).

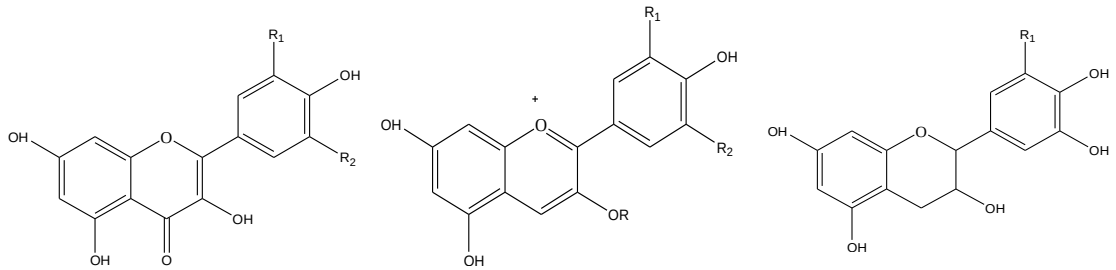


Figure 1 Basic structure of flavonoids: flavonol, anthocyanidin, and flavan-3-ol (from left to right), R can be H or OH (Jackson, 2008; Kennedy, 2008; Monagas, Bartolome, & Gomez-Cordoves, 2005). Inspection of the carbon skeleton reveals two 6-membered phenols linked by a 3-carbon chain

Non-flavonoids are located in the cell vacuoles of grape cells and are easily extracted on crushing (Jackson, 2008). Most phenolic compounds of white wine are non-flavonoids, specifically

hydroxycinnamic acids (Kennedy, 2008). Hydroxycinnamic acids have carbon skeleton of C_6-C_3 (Figure 2), hydroxycinnamic acids includes *p*-coumaric acid, caffeic acid, ferulic acid and sinapic acid (Monagas et al., 2005).

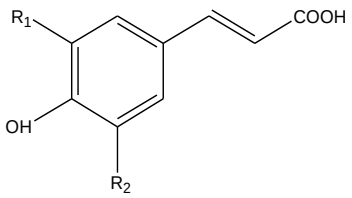


Figure 2 Typical non-flavonoid hydroxycinnamic acids, R can be H, OH or OCH3. (Margalit, 2004; Monagas et al., 2005). Inspection of carbon skeleton reveals one 6-membered phenol linked to a 3-carbon chain

The so-called 'French Paradox' is that routine consumption of red wine protects the French (on average) from the potential deleterious effects of a high fat diet from cheeses among other features of French cuisine (Frankel, German, Kinsella, Parks, & Kanner, 1993). A metabolic model to explain this paradox is that the phenolic compounds in wine inhibit the oxidation of low density lipoproteins (LDL). Oxidation of LDL is causally associated with arteriosclerosis (Jackson, 2008; Teissedre, Frankel, Waterhouse, PEFeg, & German, 1996). deLeiris, Besse, & Boucher (2010) pointed out that moderate consumption of wine with fish can increases the protection of heart. It is due to the co-operation of ethanol, phenol in wine and omega 3-fatty acid in fish.

1.1.3 Spirits

Distillation is a process for isolating and purifying materials that have similar boiling points (Zaretskii, Rusak, & Chartiv, 2010). Distillation is a secondary step after the completion of fermentation to produce spirits (Jackson, 2008). Thus flavour compounds co-distil with ethanol to provide some of the characterising flavours of brandy, whisky and gin. Ethanol, initially present in a 'wash' at between 5 and 25%, is concentrated to typically more than 60% along with co-distillates.

1.2 Fermentation

In all the above examples, beers, wines and spirits, ethanol is generated by an anaerobic fermentation. Thus, fermentation is fundamental to the creation of alcoholic drinks. In the case of

wine as the liquor relevant to this thesis, where the source of fermentable carbohydrate is grape berry sugars, mainly glucose and fructose (Margalit, 2004), these sugars are anaerobically converted into ethanol with the simultaneous production of carbon dioxide:

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

This reaction is theoretically reversible but is strongly favoured toward production of ethanol. Besides, the co-product – CO_2 – is lost as a gas from the fermentation mixture, thus precluding the reverse reaction.

Fermentation does not directly involve flavour compounds in the grape berry, so the unique character of the original grape is largely preserved (Margalit, 2004).Thus, pinot noir grapes for example and merlot grapes each yield distinct wine styles.

Fermentation takes places in the temperature range of 5 to 38°C (Margalit, 2004). White and red wines normally ferment in the temperature range of 8 to 15°C and 25 to 30°C,respectively (Jackson, 2008; Margalit, 2004).

There are two stages for fermentation: alcoholic fermentation which is carried out by a *Saccharomyces* yeast and malolactic fermentation which is carried out by lactic acid bacteria (LAB) (Bauer & Dicks, 2004; Margalit, 2004). The LAB in wine are species of *Leuconostoc*, *Pediococcus* and *Lactobacillus*, and are active after the alcoholic fermentation.

During alcoholic fermentation, most glucose and fructose convert to ethanol via glycolysis shown in Figure 3. During malolactic fermentation, malic acid converts to lactic acid (Jackson, 2008) in a decarboxylation reaction (Figure 4). Malic acid is one of the major organic acids in grape. It contributes half of the acidic taste to wine (Jackson, 2008; Redzepovic et al., 2003). Because lactic acid is less acidic than malic and citric acids (Table 1), conversion of malic acid to lactic acid decreases the acidity and thus increases the pH of the wine. Citric acid, which is also one of the major acids in grapes, is converted by LAB to lactate, acetate, acetoin and diacetyl (Jackson, 2008; Liu, 2002; Redzepovic et al., 2003). Diacetyl is an aroma compound with buttery, nutty and toasty notes (Jackson, 2008; Liu, 2002).

Because less acidic wine is more accepted by humans to the palate (Jackson, 2008), malolactic fermentations are often desirable in low pH wines, but is less useful for wines with an initially higher pH. When the pH of wine is under 3.5, *Leuconostocoenos* conducts the malolactic fermentation with no production of off-aromas. However, when the pH of wine is above 3.5, *Pediococcus* and *Lactobacillus* conduct the malolactic fermentation, but at the same time generating undesirable buttery, cheesy or milky aromas (Jackson, 2008). Above pH 3.5, the amino acid arginine is degraded to ammonia which increases the pH of wine and also increases the risk of wine spoilage (Bauer & Dicks, 2004). Thus, keeping the wine below pH3.5 during malolactic fermentation is very important. LAB also produce an exogenous esterase that hydrolyses fruity esters and can reduce the fruity aroma of wine.

Table 1 pKa value of major acids in wine (Margalit, 2004)

Acid	pKa	
	First	Second
Tartaric acid	3.04	4.34
Malic acid	3.46	5.10
Citric acid	3.13	4.74
Lactic acid	3.86	

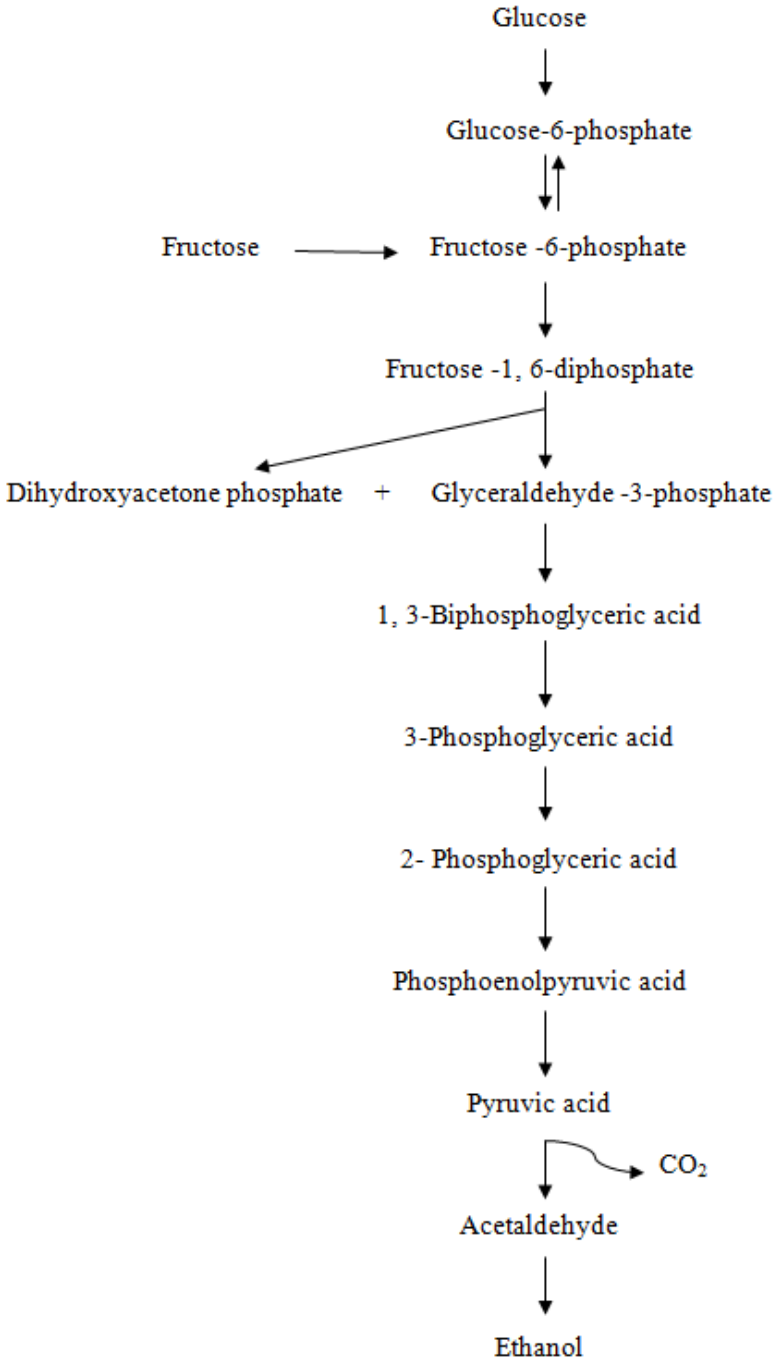


Figure 3 Production of ethanol from glycolysis

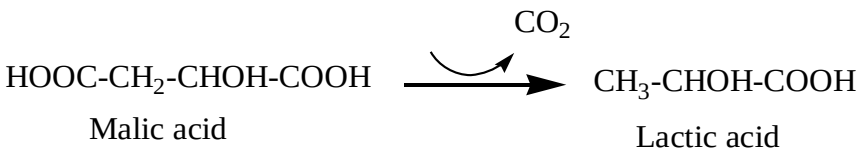


Figure 4 Malolactic fermentation

1.3 Ageing of alcoholic drinks

There is a series of adjustments made to wine before bottling wine based on the post ferment quality of the wine. These adjustments are acidification, deacidification, blending, colour adjustment, stabilisation and ageing, that are variously applied to different wines. Ageing is one of the important steps of post fermentation treatments (Jackson, 2008).

There are two stages of ageing: maturation and reductive ageing. Maturation is defined as the change occurs between alcoholic fermentation and bottling. Maturation generally takes 6 to 24 months but some wine might take more than10 years (Jackson, 2008). It is typically done in closed oak barrels where some oxygen will contact the wine from diffusion through the oak wood. During the maturation, a malolactic fermentation may be applied. In the reductive ageing, wine is stored in the bottle with minimal oxygen diffusion through the cork (Jackson, 2008). Stelvin caps, in common use in Australasia, admit diminishingly small quantities of oxygen.

1.3.1 Chemical reactions during ageing

Wine colour changes during the ageing: light yellow shifts to deeper yellow, gold and brown in white wines and violet red changes to tawny red in red wines (Domine, 2004; Jackson, 2008; Margalit, 2004).When wine is aged, the fruity character fades and more subtle flavour and a smooth texture develops (Jackson, 2008). The literature reports many reactions that are responsible for these changes. A non-exhaustive summary of these reactions follows.

Important chemical reaction during ageing include phenolic oxidation and polymerization (Margalit, 2004). Phenolic oxidation is either enzymatic or non-enzymatic, the former being more important in wine. The mechanism for the enzymatic oxidation of phenolic acid is shown in Figure 5. In the presence of oxygen and the enzyme polyphenoloxidase (PPO), orthodiphenols are converted to diquinones and hydrogen peroxide. Laccase is another enzyme that oxidises phenols in wine, but is less important than PPO.

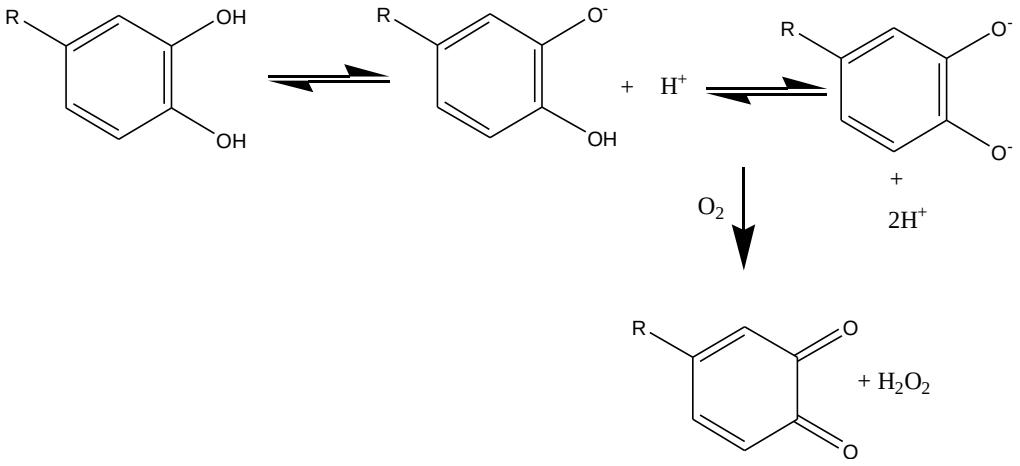


Figure 5 Oxidation of phenolic compound (Margalit, 2004)

For red wine, decreased colour intensity and browning result both from a disassociation of non-covalent anthocyanin complexes and progressive formation of anthocyanin-tannin covalent polymers. These changes decrease the 520/420 nm absorbance values (Jackson, 2008). High 520/420 nm values indicate a bright red colour whereas low values indicate a shift to a shade of red brick, often called tawny red. Tannins in wine can also co-polymerise (Figure 6). In both these types of complexes, the bitterness and astringency of wine decreases. Blueness increases with the number of free hydroxyle group, while redness increase with the degree of methylation.

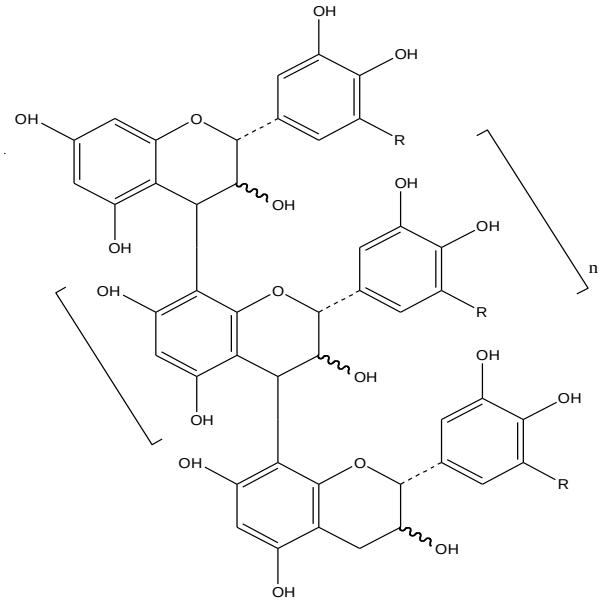


Figure 6 Structure of procyanidin polymers-condensed tannin (R= H, OH), with n = 0,1,2 etc. (Jackson, 2008)

Another polymerization reaction starts with the metal catalysed oxidation of tartaric acid to

glycolic acid. Glycolic acids bind to catechins and possible other phenolics, creating complexes that in turn can polymerise with one another (Jackson, 2008; Margalit, 2004). The reaction generates both colourless and yellow polyphenols.

The esters contribute to wine with either fruity, soapy or lard-like aroma depending on the length of hydrocarbon chain of the acid part of the molecule. The longer the chain, the less fruity the character (Jackson, 2008).

With time, concentrations of esters change slowly during ageing to establish chemical equilibrium (Sumby et al., 2010). Hydrolysis and synthesis of various esters happen simultaneously. Generally, ethyl esters of straight chain fatty acids, and fusel alcohol acetates decrease in concentration through hydrolysis (Peinado & Mauricio, 2009; Sumby et al., 2010). In contrast, ethyl esters of branched short-chain fatty acids such as ethyl isobutyrate, increase in concentration (Diaz-Maroto, Schneider, & Baumes, 2005). (Ethyl isobutyrate is important in muscadet flavour.) Ester of dicarboxylic acids, diethyl succinate for example, also increase during ageing (Câmara, Alves, & Marques, 2006; Jackson, 2008). Branched fatty acid ethyl esters are less volatile than their straight-chain analogue (Sumby et al., 2010).Overall, fruity ester aromas decrease during ageing and this is clearly evident in older wines.

In reductive ageing, oxygen is nominally absent, being impermeable through glass. However, some oxygen is trapped during the bottling process, and where corks are used to seal bottles, some oxygen will slowly diffuse into the wine. The main reactions are the phenolic oxidation and acetaldehyde formation (Margalit, 2004). As seem in the Figure 7, oxygen reacts with phenolic compounds to produce hydrogen peroxide which will react as an oxidation agent of ethanol to produce acetaldehyde. The concentration of formed acetaldehyde will reduce when it reacts with sulphur dioxide or sulphur dioxide reacts with phenol. The concentration of sulphur dioxide decreases as ageing processing.

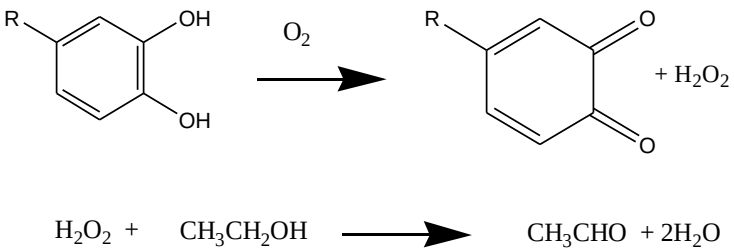


Figure 7 Formation of acetaldehyde from ethanol from Margalit (2004)

Dimethyl sulphide (CH_3-S-CH_3) is one of the major compounds found in the aged wine. At low levels dimethylsulphide contributes asparagus odour, fruitiness to wine. As its level increases, it contributes cooked cabbage, black olive, and truffle sensory characters to wine. Its content increases during reductive ageing. It was found that there is significant correlation between dimethyl sulfide and maturation bouquet development for Riesling wine (Margalit, 2004).

1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) is a 13- carbon compound that is found in aged white wine. It contributes a spicy jet fuel-like volatile note to wine especially to Rieslings. It is not found in the grape juice or young wine, but its concentration increases during aging as result of hydrolysis of precursors of TDN such as 2,6,10,10-tetramethyl-1-oxaspiro(4,5)-dec-6-ene-2,8-diol (Figure 8) (Margalit, 2004). It was found that the concentration of TDN in Riesling increases with the age of maturation.

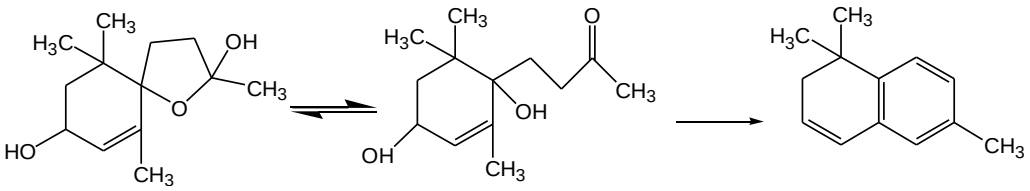


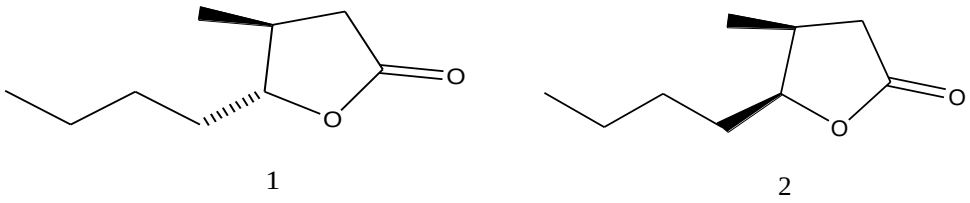
Figure 8 Production of TDN (Margalit, 2004)

Carbohydrate degradation during ageing produces caramel-like chemicals such as 2-furfural, 2-acetylfuran and ethyl-2-furoate. The concentration of these compounds increases during ageing (Jackson, 2008).

The concentration of terpenoid increases during ageing (Margalit, 2004).

1.3.2 The role of oak in ageing alcoholic drinks

Traditionally, wine is matured in oak barrels, the inside of which is flame charred to a greater or lesser extent. Such barrels are described as 'toasted'. The filled barrels are stored in a cool environment, e.g. a cellar, where the ambient temperature is always less than 20°C and unvarying beyond a few degrees centigrade. Some water and ethanol evaporate during storage of wine in barrels, which creates slight vacuum environment above the wine. This evaporation increases the wine's flavour (Gore, 2007; Margalit, 2004). Some oxidation takes place after oxygen is unavoidably included during barrel racking and topping up of lost volume. During barrel ageing, many compounds are extracted to the wine, and oak toasted to the point of charcoal formation will extract compounds from the wine (Young, Kaushal, Robertson, Burns, & Nunns, 2010). The cis and trans forms of oak lactone, methyl octalactone, are internal esters of 4-hydroxyoctanoic acid (Figure 9).



1 2

Figure 9 Structure of oak lactone isomers, 1 is trans and 2 is cis (Pollnitz, Jones, & Sefton, 1999)

Oak lactones are important oak volatile compounds and contribute woody, coconut and oaky flavours to the wine (Jackson, 2008; Moreno-Arribas & Polo, 2009). It is an important compound in any alcoholic drink aged in oak, and that includes whisky, brandy, some tequilas and rums, and wine.

Oak wood also contains hydrolysable tannins such as gallitannins (polymers of gallic acids with glucose) (Figure 10) (Hartzfeld, Forkner, Hunter, & Hagerman, 2002) and ellagitannins (polymers of ellagic acids with sugars) (Khanbabaee & van Ree, 2001).

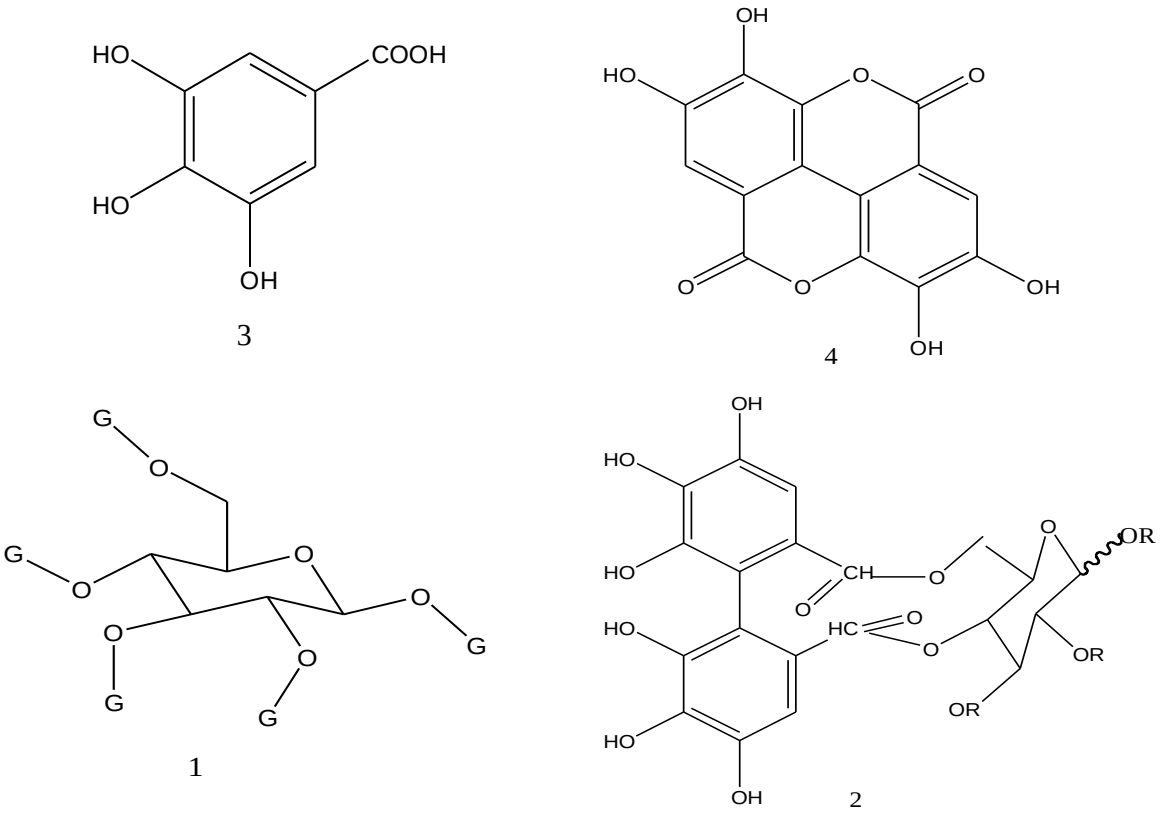


Figure 10 Structure of gallitannins (1), and ellagitannins (2), gallic acid (3) and ellagic acid (4). R is substitute and G is gallic acid

During the storage, these hydrolysable tannins are extracted from the barrel and the astringency of wine increases.

As with all woods, lignin is major part of oak wood. On toasting, aromatic aldehyde components such as vanillin (vanillaldehyde), guaiacol, 4-methyl guaiacol, eugenol are heat-generated from lignin (Jackson, 2008), are they are extracted into alcoholic drinks. The extracted aromatic aldehydes increase the complexity of wine: vanillin has vanilla flavour, eugenol has spicy clove-like flavour, whereas guaiacol and 4-methyl guaiacol have smoky flavours (Margalit, 2004).

In the past few decades, oak chips have being used in wine maturation in place of barrels. In this situation, oak is put into wine as opposed to wine into oak. This represents a major cost saving, because oak chips are greatly cheaper than barrels. Depending on the surface area of oak chips exposed to wine, the extraction of flavour from oak chips can reach the recognition threshold within a few hours. The oak chips from different species yields different amounts of phenolic compounds in the wine (Young et al., 2010). But oak chips can only replace the extraction role of barrel ageing

(Margalit, 2004),aeration has to be managed with the use of oak chips to ensure a subtle controlled oxidation (Margalit, 2004).

1.3.3 Costs of ageing alcoholic drinks

Overall, ageing of spirits, and of wines to a point, improves drinking quality. The point in time for wine ageing (Edwards & Spawton, 1990; Jones & Storchmann, 2001) depends on very many factors, such as vintage, alcohol content, pH, residual sugar, exposure to oak. Red wine typically takes two years to age, and fortified wine much longer. For example, the average ageing time for sherry is about 15 years from the solera system. Vinjaune is aged in barrel at least 6 years. Madeira wine may be aged in the oak for a minimum of 20 years plus two years in the bottle (Câmara et al., 2006; Jackson, 2008).

Ageing is one of the significant costs in wine production (Edwards & Spawton, 1990). New French oak and American oak barrels imported into Australia for example, cost about A$1100 and $700, respectively (Gore, 2007). Then there are the costs of storage that also extend to financing costs, because unless the wine is sold *en primeur* the costs of ageing must be borne by the producer. These costs can force the winemaker to sell the wine at a lower price before wine reaches its peak quality in order to achieve cash flow. Therefore, shortening the ageing process without detracting from wine quality is useful in the wine industry.

1.3.4 Methods of reducing ageing time for wines

The storage temperature influences the rate of ageing because heat increases the rate of most reactions involved in ageing (Jackson, 2008). Wine stored at a low temperature such as 10°C takes ten times longer than wine stored in high temperature such as 40°C to reach the same age development. For example, it was found that the concentration of TDN increases from when the ageing temperature increases from 15°C to 30°C (Jackson, 2008). Ten degrees centigrade is the traditional cellar temperature that allows the retention of most fruit esters while keeping other ageing reaction, but even 20°C storage does not cause adverse sensory changes to wine. However, if the ageing temperature is higher than 40°C, carbohydrates in the wine undergo Maillard and thermal degradation reactions that result in a brown wine and caramel flavour.

Wines are normally stored in areas free of vibration. Jackson (2008) states that “vibration is

commonly considered to disrupt or accelerate ageing", but did not elaborate this claim. The claim is not unreasonable however.

Ultrasonic waves can 'reconstruct' molecules (Suslick, 1989). When ultrasonic waves were applied during wine fermentation, formation of ethanol and iso-amylacetate was accelerated (Matsuura, Hirtotsune, Nunokawa, Satoh, & Honda, 1994). A comparison of rice wine treated by 20 kHz ultrasonic waves after fermentation and rice wine that was aged under traditional method, showed that their sensory attributes, pH, major alcohols and esters concentrations were very similar (Chang & Chen, 2002).

Strong electric fields have been proposed as a method of accelerating improvement of wine quality. An electric field is fundamentally a charge difference between two points, and in wine applications is applied as a field across a gap through which the wine or other food is stationary or flows enclosed in, say, a glass tube.

Pulsed electric field (PEF) is a treatment involving the application of microsecond pulses of a high electric field (kV cm^{-1}) through a material located between two electrodes (Puértolas, López, Condón, Álvarez, & Raso, 2010). Lopez, Puértolas, Hernandez-Orte, Alvarez, &Raso (2009) found that wine produced from Cabernet Sauvignon grapes treated by PEF before maceration and fermentation contained higher anthocyanins concentrations (such as malvidin-3-glucoside and malvidin-3-glucoside acetate) and a higher phenolic index as measured by absorbance at 280 nm. In that experiment, the crushed grapes were treated in a flow-through parallel-electrode treatment chamber with an electrode gap of 1 cm and an area of 20 cm^2; the pulsed electric field treatment was 50 pulses of 5000V cm^{-1} [2]at 122 Hz. In another PEF experiment (same grape variety and same conditions), the properties of wines were investigated during ageing in oak barrels and after bottling. Wine produced from PEF-treated grapes contained higher concentrations of anthocyanins, flavan-3-ols and hydroxycinnamic acids than wine produced from untreated grapes (Puértolas, Saldaña, Álvarez, & Raso, 2010; Puértolas, Saldaña, Condón, Álvarez, & Raso, 2010)

[2] These electric values are as reported, but could mean peak voltage or voltage root mean square (Vrms). In the experiments, voltages are expressed as Vrms.

In a Chinese publication with only an abstract in English, Chen, Zeng, Dong, & Yang (2004) found that an alternating fields of 2000 V cm^{-1} and 5000 V cm^{-1} generated higher free amino acid concentrations in red wine. Further information could not be obtained from this limited publication. In another Chinese publication with an English abstract (Zeng, Fu, Li, & Yu, 2004), unaged rice wine was treated with a 40,000 V m^{-1} 50 Hz electric field for 180 min. The treated wine showed a similar 1H NMR spectrum to a control wine aged by traditional methods. Both had a different spectrum from unaged wine. It is emphasised that the value 40,000 V m^{-1} was as reported in the abstract, noting that all other publications encountered were expressed in cm^{-1}.

In an English language publication, Zeng, Yu, Zhang, & Chen (2008) reported that Cabernet Sauvignon wine treated with an alternating field of 600 V cm^{-1} at 3000 Hz contained higher concentration of esters, free amino acids and lower alcohol concentration than control wine. There was a sensory improvement in the treated wine according to 12 experienced tasters. However, according to Zeng et al.(2008) the changes of chemical compounds that were measured were not sufficient to explain the marked sensory changes. In short the reason for the change in sensory properties remained largely unexplained.

Based on the above reports, Dr Sadhana Talele of University of Waikato, Department of Engineering, constructed a flow through 'wine aging machine' (WAM) modeled on the design by Zeng et al.(2008). In their design, the wine flows in a sinuous glass tube between two aluminium plates across which the voltage is applied (Figure 11). Other details are described in Chapter 2.

The results with WAM have been reported by Talele, Benseman, & Scott (2013), using red wines (Pinot Noir). They confirmed that electric field technology can rapidly age wine. The flow through WAM constructed by the University of Waikato was the first of the models used in this study, but was complemented with a static WAM, which is described in Chapter 2. White and red wine were both treated by the WAMs and the outcomes were evaluated by a number of methods.

1.3.5 Physical and chemical evaluation methods of WAM-treated wine

Spectrophotometry is a technique to determine the colour of transparent liquids, solids and gases, but mostly applied to liquids. Absorbance is defined as $A = \log \frac{P_0}{P}$ (Harris, 2003), where P_0 is radiant power incident on the sample, and P is the radiant power transmitted by the sample.

Absorbance is directly proportional to the concentration, c, of the light absorbing species in the sample (Sherman Hsu, 2000). In a relationship known as Beer's law, $A = \varepsilon bc$, where c has the units of mole per litre (M), b is the pathlength of light across the sample, and is commonly expressed in centimetres. The quantity (epsilon) is called the molar absorptivity and has the units $M^{-1}cm^{-1}$ to make the product εbc dimensionless, as expected from the ratio $\log \frac{P_0}{P}$. Molar absorptivity is the characteristic of a substance that tells how much light is absorbed at a particular wavelength by a molar solution (Harris, 2003). However, it is important to note that molar solutions are unlikely to be used in spectrophotometry because molar solutions of coloured chemicals are so intensely coloured that diminishingly low amounts of transmitted light reach the detector, so that the ratio $\log \frac{P_0}{P}$ becomes unreliably large. Therefore instrument errors are minimized if the absorbance falls in the range A= 0.4 to 0.9 (Harris, 2003).

High performance liquid chromatography (HPLC) is a popular technique used for resolving the chemical composition of complex solutions like wine. The main components of the HPLC system are the pump, injector, column, detector and data system (Nielsen, 2000). The liquid containing the solutes of interest is injected onto a column that contains a so-called stationary phase. The solutes are adsorbed onto this phase. A mobile phase (the solvent) is pumped under high pressure through the stationary phase and competes with solute molecules for adsorption sites on the stationary phase. Elution occurs when the solvent displaces solute from the stationary phase (Harris, 2003), and this happens at different rates for different solutes. Solvent gradients are particularly useful to exploit chemical differences between the solutes in solution. Detection of the eluates can be achieved by a number of techniques including refractometry, spectrophotometry, and mass spectrometry. A mass spectrometer (MS) measures the masses of atoms or molecules or fragments of molecules in a sample (Harris, 2003). To obtain a mass spectrum after liquid chromatography, the progressive eluates from the column are ionised by an ionisation source in a high vacuum. The resulting ions are accelerated by an electric field and separated according to their mass-to-charge ratio (m/z). The signals of separated ions are detected and the data recorded (Ashcroft, 2013; Barker, 2000; Harris, 2003). Contemporary mass spectrometers can analyse negative and positive ions, and these two techniques are described in more detail in Chapter 3. High pressure is fundamentally

important to HPLC analysis because high resolution depends on completing the chromatography before the solutes can diffuse from the adsorption sites. Higher pressures are therefore better for separation. Pressure used in ultra performance liquid chromatography (UPLC) can reach 1000 bar, but pressure used in HPLC can reach 400 bar (Roge, Firke, Dhane, Gunjkar, & Vadvalkar, 2011). Smaller particles (below 2 μm) and higher velocity of mobile phase are used in UPLC (Roge et al., 2011; Swartz, 2005). Thus ultra performance instruments (UPLC) are more analytically powerful than HPLC equivalents for separation (Ashcroft, 2013). HPLC and UPLC were both used in this research.

1.3.6 Sensory evaluation of WAM-treated wine

People perceive foods in the aspects of appearance, smell, texture and taste. These perceptions are stimuli. For the stimuli to trigger responses, a chain of events unfolds: a stimulus interacts with the sense organ and is converted to a nerve signal which travels to the brain. The brain interprets and organises the incoming signal with respect to previous experience in the memory, and becomes a response (Meilgaard, Civille, & Carr, 1999). Different people can and often do respond differently to the same stimulus. In the case of foods, this variability between people can be overcome by replication for statistical purposes (Meilgaard et al., 1999), and has proved to be commercially useful in the food industry. Thus sensory evaluation is widely used in quality control and product development.

The basic triangle test is one of the sensory tests to discover whether a perceptible difference exists between samples of two treatments. The statistical analysis is made under the tacit assumption that only the α-risk matters. An α-risk is defined as the probability of concluding that a perceptible difference exists when one does not. An α-risk of 0.1to 0.05 indicates moderate evidence that a difference is real, an α-risk of 0.05 to 0.04 indicates strong evidence that a difference is real, and an α-risk of 0.01 to 0.001 indicates very strong evidence that a difference is real (Meilgaard et al., 1999).

In the triangle test, three coded samples are presented to panellists. Two samples are identical and the other is different. Panellists, the more the better, are asked select the odd sample of the three. The number of panellists who made the right decision is counted and the data are interpreted

with Table 2 (see Chapter 2) that is based on the α-risk (Meilgaard et al., 1999).Triangle tests normally employ 20 to 40 panellists. There are six ways for presenting sample to panellists, which are: AAB, ABB, ABA, BBA, BAB and BAA. The order of presenting samples to panellists is randomised.

A triangle test is very effective in situations where treatment effects may have produced product changes that are difficult to characterize by one or two attributes (Meilgaard et al., 1999). This is the situation likely to be encountered in the present research, which is why this test was chosen. More details are supplied in Chapter 2.

Chapter 2

Materials and Basic Methods

2.1 Choice and storage of wine

A single white wine was chosen. It was an Astrolabe Marlborough Sauvignon Blanc 2010. Twenty four bottles were bought at retail in October 2011 and stored on their side in the dark at 5°C. The intention was to minimise bottle ageing over the course of the research, nominally one year. Whole bottles were always used for experiments.

Several cheap red wines were bought at various times from a Countdown supermarket in Auckland and stored on their side in the dark at room temperature before use. These were an EskValley Merlot Cabernet Sauvignon 2009, a Hardy's Carbernet Merlot, a Banrock Station Cabernet Merlot 2011, and a Mill Road Pinot Noir 2011.

2.2 Description of the wine ageing machines

There were two basic designs. The flow-through wine aging machine (WAM) is shown in Figure 11. Three horizontal aluminium plates measuring approximately 500 mm by 200 mm were maintained 7.5 mm apart, allowing 18 m of 7 mm diameter glass tubing to loop backwards and forwards through out the two gaps (Figure 11). The internal diameter of the tubing was 4 mm (Talele et al., 2013). A volume of wine was poured into the reservoir that led to silicone tubing that in turn led to the glass tubing. The alternating voltage was applied to the plates according to the need. The equipment was considered to be safe for the operator because the plates were protected from human contact by a horizontal Perspex shield, and the equipment was all insulated by a wooden box (Figure 12). The speed of wine flow in the WAM was controlled by either peristaltic pump (positive pressure) or vacuum (negative pressure).The means of inducing flow is not shown in Figure 11. Gravity alone did not induce flow. Two peristaltic pumps were used, a unknown brand and a Gilson's Manipuls 2 peristaltic pump. Details of flow rates are presented in Chapter 3.

Although there was only one flow-through WAM, there were two electronic configurations. The first configuration was as received from the University of Waikato and used a peristaltic pump

applied across the silicone tubing. The electronic configuration involved in powering the high voltage plates with a frequency generator power amplifier and a high voltage transformer. The generator-amplifier combination supported a frequency range from 10 Hz to 195 kHz. The amplifier was a MOSFET- based class AB amplifier capable of delivering around ±50 V peak current of about 10 A and a nominal gain of about 30 dB. The transformer was able to generate voltage outputs from 50 V peak to over 2000 V peak (Talele et al., 2013).

To clean the WAM, 1500 mL of deionised water was poured into the reservoir that led to silicone tubing that in turn lead to the glass tubing.

This configuration allowed independent variation of voltage and sinusoidal frequency. The ranges were 0 to 3000 Hz and 0 to 1000 V peak (= 707 V rms). However, this design was prone to catastrophic failure, as happened in the first three months of work. A second design by Mr Brett Holden of AUT University was limited to 3000 Hz, but the voltage could be varied from 0 to 1000 V peak.

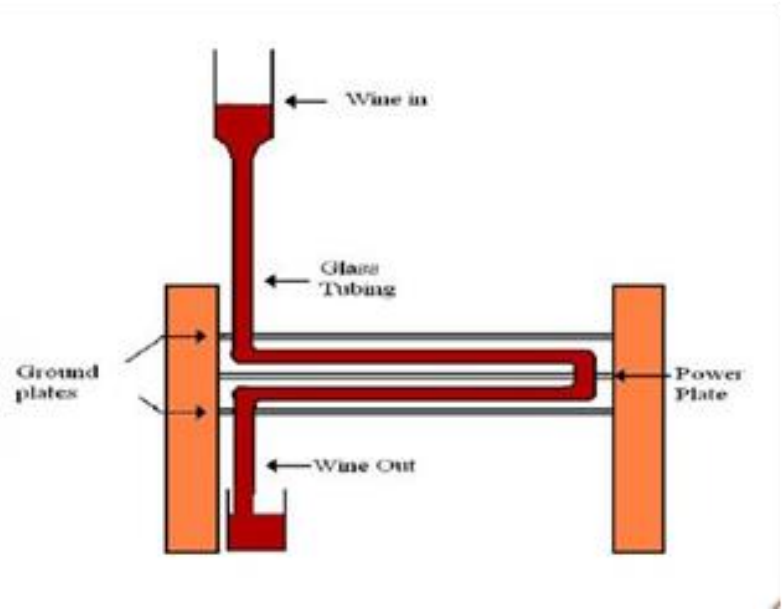


Figure 11 Flow diagram of the flow-through WAM (Talele et al., 2013). The means of forcing the wine through the glass tubing is not shown in this figure

Figure 12 The flow through WAM. In this (second) configuration, the wine was pulled through the tubing by vacuum. For the first configuration the peristaltic pump (not shown) was located across the silicone tubing just visible below the reservoir

The static wine aging machine (WAM) comprised two vertical aluminium plates (240 x 180 mm) held 15 mm apart. A rack able to hold twelve 11 x 150 mm test tubes was inserted into the gap and the test conditions were applied as required (Figure 13). The rack also triggered a micro-switch such that power could not be applied without rack insertion. This was a safety feature.

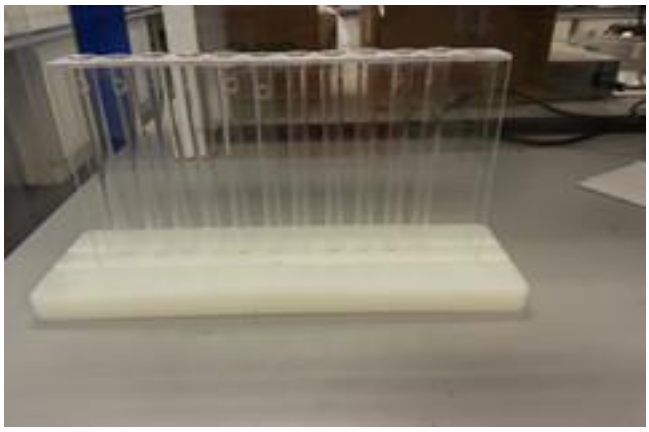


Figure 13 The rack for holding test tubes

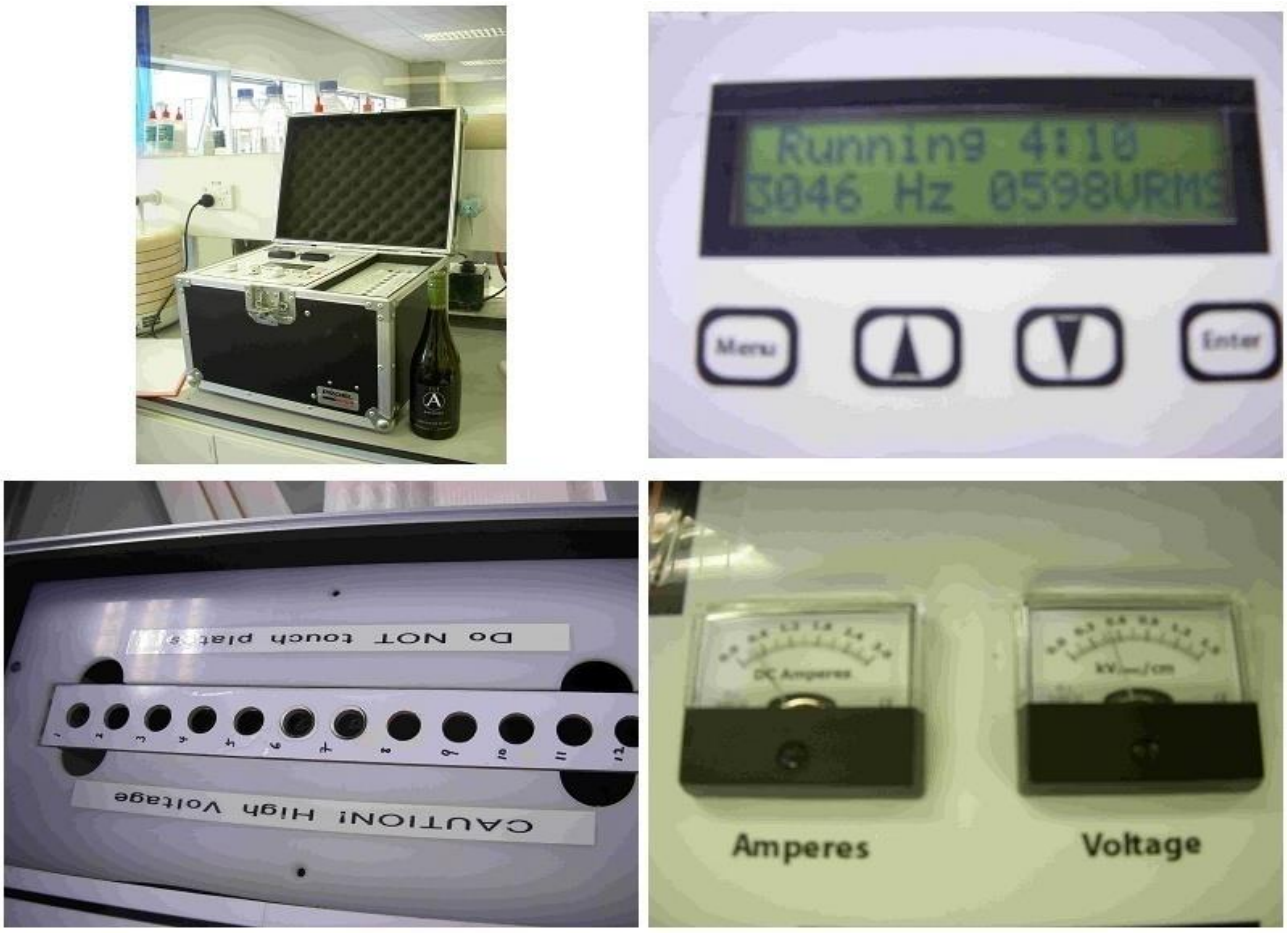


Figure 14 The static WAM. Test tubes containing the liquids under study are held in the numbered rack (lower left), and the frequency and voltage are adjusted by the digital control (top right)

2.3 Validation of frequency and voltage

It was obviously important to be sure that the voltage was applied to the plates when wine was subjected to the WAM treatments. The voltage meter shows that a potential difference is being generated, but does not necessarily mean that the potential difference is applied to the plates. It could be applied only to the wires leading to the plate. This could be tested routinely by applying a voltmeter across the plates. However, for safety reasons this was not routinely feasible given that up to 1000 volts (peak) were often generated.

One critical test of connection was sound. Frequencies up to 3000 Hz are in the audio range. The strength of electric field of the static WAM was set at 299 Vrms cm^{-1}, the frequency was adjusted from 1200 Hz to 4145 Hz at about 500 Hz intervals. In another test, the frequency of the WAM was set at 1200 Hz, the strength of electric field was adjusted from 299 Vrms cm^{-1}to1205 Vrms cm^{-1} at

200 Vrms cm^{-1} intervals.

Another test involved capacitance and current and was applied to the second flow-through WAM. A high pitched hum was generated when the electric field was on. The argument was this: if the wires were attached to the plates, a small but measureable current should be generated because charging the plates involves a greater movement of charge (current) than if the wires were disconnected. This is because the wires have negligible capacitance compared with the plates. Indeed, both forms of the WAM have ammeters (Figure 12 and Figure 14).

2.4 Spectrophotometers

An GE Amersham Ultrospec 2100 Pro UV/Vis spectrophotometer (Figure 15) has a 190 to 900 nm range. Its spectral bandwidth is less than 3nm and the absorbance range is –3 to 3. The photometric reproducibility is within 0.5% of absorbance value to 3 at 546 nm. The xenon lamp is the light source and dual silicon photodiodes are used for detection.

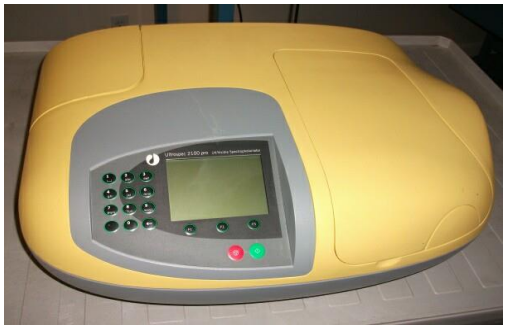


Figure 15 An GE Amersham Ultrospec 2100 Pro UV/Vis spectrophotometer

A GE Healthcare Ultraspec 7000 UV/Visible spectrophotometer (Figure 16) has a 190 to 1100 nm range and –4 to 4 absorbance range. The photometric reproducibility is within 0.2% at an absorbance value of 1, at an unspecified wavelength. The spectral bandwidth is 2nm. It uses pulsed xenon lamp as light source and dual-silicon photodiodes as detectors.

Figure 16 A GE Healthcare Ultraspec7000 UV/Visible spectrophotometer

2.5 pH determination

With a calibrated pH meter from Radiometer Copenhagen, PHM201, the pH of aliquot was measured, the pH meter was rinsed with Milli Q water between aliquot measuring.

2.6 Measurement of light absorbance

As discussed in more detail in the Introduction, it was proposed that if an electric field were to age wine rapidly then changes are likely to occur in the phenolic compounds that are plentiful in win, red wine in particular. Phenolic compounds absorb strongly in the ultraviolet (UV) range, so interest was focused on this range (200 to 400 nm for white wine) although the full range to 700 nm was used for red wine. White wine (diluted as always) showed negligible absorbance in the visible range.

Wines were diluted substantially before absorbances were measured. Why was this done? The dynamic range of both spectrophotometers is 0 and 3, but wines in a 10 mm cuvette have absorbances orders of magnitude higher, particularly red wine. Only negligible amounts of light reach the detector. Moreover, departures form Beer's law are minimised if the absorbance falls in the range 0 to 1. Thus wine has to be diluted (Young and others, 2010), and experience has shown that 1:20 dilution of white wine with water or other aqueous diluent generates useful information. Red wines had to be diluted 1:100.

One common procedure for measuring the absorbance differences between control and treated wine was as follows. A control wine was accurately diluted 1:20 with water[3] and used to set a zero baseline between 200 and 700 nm, using a 10 mm quartz cuvette. Then the absorbance of WAM treated wine was measured vs the baseline. This technique showed positive and negative differences, but never absolute absorbance value. In the negative case, the treated wine could also be used to set a zero baseline.

Another procedure, used later in experiments, was to set the baseline with Milli Q water using a 10 mm quartz cuvette. The absorbance of control wine and WAM treated wine was then measured. In this case, the absolute absorbance value was obtained after correction for dilution.

It must be pointed out that no observable changes in UV absorbance does not necessarily mean that the wine was unchanged by an electric field. Thus if aliphatic esters were hydrolysed for example, there would be no change in UV absorbance. UV absorbance was just one measure of potential change.

2.7 Sensory evaluation

The wines were evaluated by differing groups of between 22 and 30 AUT students and staff, who were all untrained, although many were wine drinkers. The wines were evaluated for colour and aroma together in a triangle test, where the panellist is asked to pick the odd glass out where two glasses contained the same wine and the third the other condition. The wines were marked with three-digit random numbers between 0 and 999. There are two ways of presenting the glasses: treated, treated, control and control, control, treated, randomised in both cases for physical position on a tray. In applying the test to different experiments, the two ways of presentation were alternated. This was not the prefect method of randomisation, but the use of only three glasses rather than six for each experiment minimised the possibility of error.

Each of the three 200 mL tulip-shaped tasting glasses were numbered, 100 mL volumes were poured, and the glasses covered with a watch glass. A glass of water was also presented (Figure 17),

[3] The quality of the water used for different experiments is described in the Results and Discussion section.

and served to refresh panellists' sense of smell. The order of glasses was randomised for each panellist all of whom were asked the simple question (Figure 18). Each panellist had to smell the water before smelling the next sample. For a selection outcome to be statistically significant, more than 1/3 of panellists had to correctly identify the odd one out. Clearly the fewer the panellists the greater the number of correct identities required to achieve significance (Table 2).

Figure 17 Presentation of wine to panellists

Please circle the one which is different to the other two (aroma, appearance)

495 497 499

Figure 18 The question asked of panellists

Table 2 Statistical tables for the triangle tests

Number of trials (panellists)	Correct answers to show is a difference ($P < 0.05$)	Correct answers/number of trials	Correct answers to show is a difference ($P < 0.01$)	Correct answers/number of trials
6	5	0.83	6	1.00
8	6	0.75	7	0.88
10	7	0.70	8	0.80
12	8	0.67	9	0.75
14	9	0.64	10	0.71
16	9	0.56	11	0.69
18	10	0.56	12	0.67
20	11	0.55	13	0.65
22	12	0.55	14	0.64
24	13	0.54	15	0.63
26	14	0.54	15	0.58
28	15	0.54	16	0.57
30	15	0.50	17	0.57
32	16	0.50	18	0.56

2.8 Liquid chromatography-mass spectrometry

Two types of liquid chromatographs were used. One was an Agilent 6550 iFunnel Q-TO Ultra performance liquid chromatograph- mass spectrometry (UPLC-MS). Another one was an Agilent 6420A triple quadrupole mass spectrometer (high pressure liquid chromatograph HPLC-MS/MS). They are fully described in Chapter 3. LC-MS is the first stage of LC-MS/MS. It separates the compounds. LC-MS/MS includes a second filtering process that separates the parent ions into smaller fragments. This is more useful for identifying compounds than simple MS (Baynham, 2006).

2.9 Outline of experiments performed

After establishing the performance of the peristaltic pump and confirming that charge was

applied to the plates as indicated by meters, experiments are described beginning with treatment of the wine with WAM equipment in various combinations of strength of electric field, frequency and time. The measured outputs were colour in the ultraviolet range, sensory discrimination tests, and data from liquid chromatography-mass spectrometry.

Chapter 3

Results and Discussions

3.1 Calibration of flow rates

Figure 19 and Figure 20 show the calibration of the two peristaltic pumps. To do this 100 mL volumes of deionised water were passed through the previously empty tubing over a wide range of pump speeds. The times taken for the volumes to emerge were recorded. A similar method was adopted for the negative pressure (vacuum) method of inducing flow.

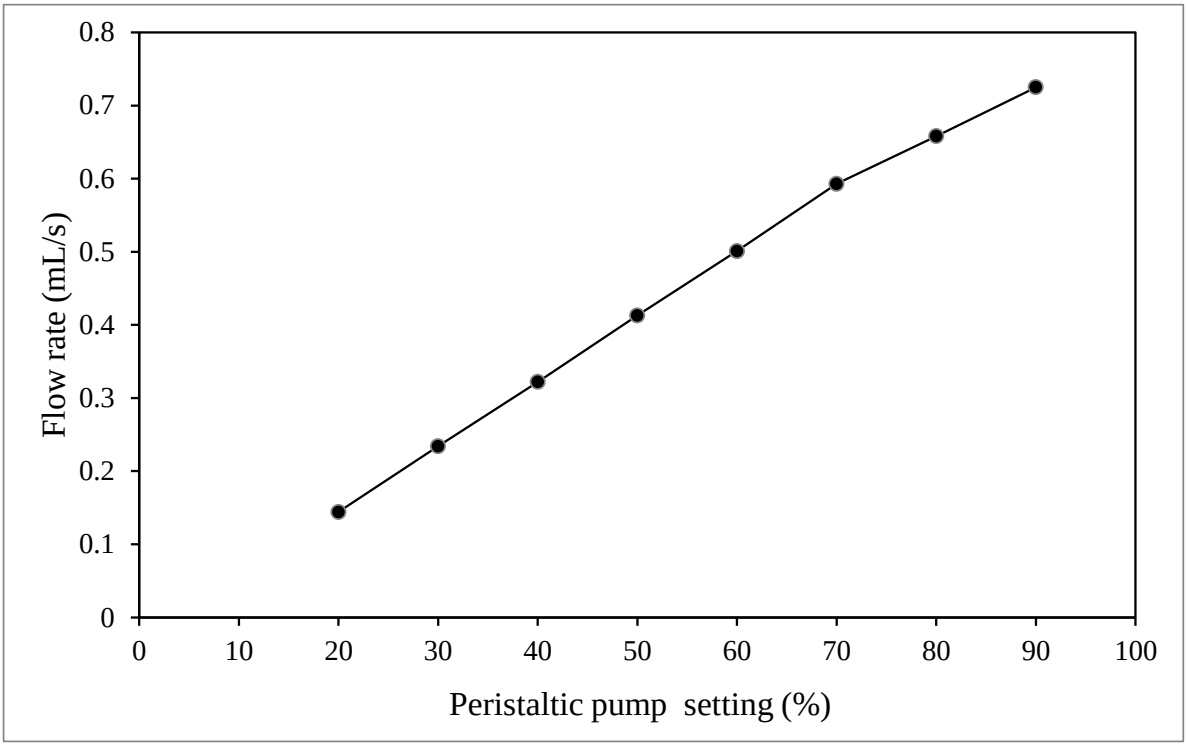


Figure 19 Calibration of the unknown brand peristaltic pump

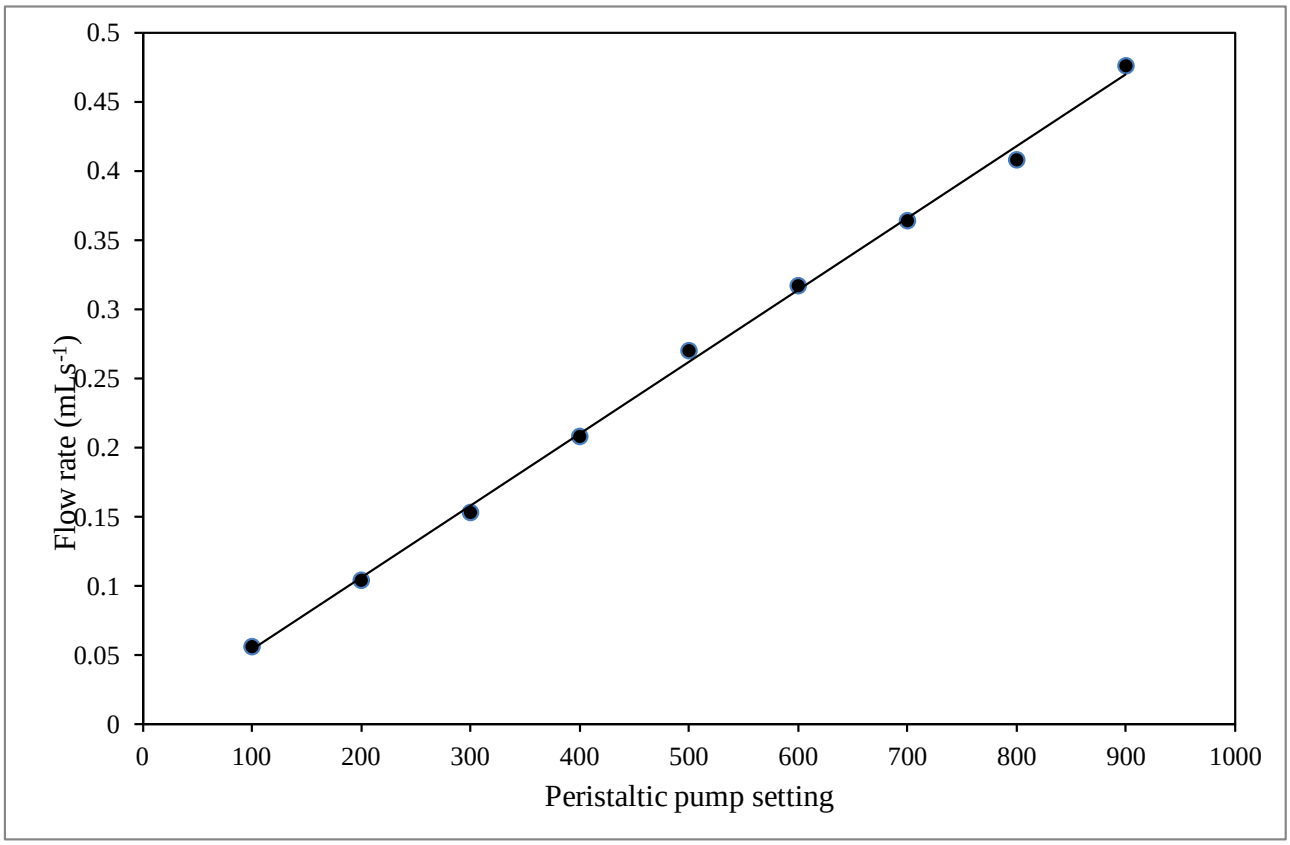


Figure 20 Calibration of Gilson's Manipuls 2 peristaltic pump

3.2 Pilot experiments

3.2.1 Introduction

For reasons outlined in the Basic Methods (Chapter 2), the Sauvignon Blanc wine was chosen for this work.

3.2.2 Methods

For no particular reason the speed of peristaltic pump of the flow-through WAM was initially set at 90% meaning that the residence time of wine in the electric field was minimal. The electric field was applied and 250 mL of wine was poured into the separation funnel at the top of WAM, the plumbing of which had previously been cleaned with deionised water and drained by the peristaltic action to be substantially but not perfectly dry. The wine flow was started by opening the valve at the base of the separation funnel. When the wine emerged at the exit, the first 30 mL was discarded in the knowledge that it would be slightly diluted by residual water in the plumbing. The collected wine was designated '90% Test 1'. This initial 250 mL of electrical field-treated wine was followed by another 250 mL (90% Test 2), and was in turn followed by 90% Control

where the field was not applied (Table 3).

Subsequently, the cycle was repeated for longer residence times, with the peristaltic pump set to 50% and 20% (Table 3). The pH of each treatment was measured after passage through the WAM and the temperature was also recorded. After each of these treatments, the recovered wine was immediately distributed among vials (about 30 mL) with Teflon cap liners. The vials were filled to overflowing so as to exclude air, and were stored at 4°C until required for spectrophotometry that was usually done within two days.

This basic experimental design was repeated with one minor difference: an additional control was performed, generating 12 retained vials rather than 9.

As described in Chapter 2, dilutions were made 1:20 with deionised water and the absorbances determined by difference where each speed test had its own control, C, G or K. The absorbance values were corrected for dilution by multiplying the raw data by 20.

Table 3 Pilot experiments with the flow-through WAM, showing code letters A, B etc. to identify treatments

Treatment								
High flow (90%) 0.73 mL s^{-1}			Medium flow (50%) 0.41 mL s^{-1}			Low flow (20%) 0.14 mL s^{-1}		
Treated A	Treated B	Control C	Treated E	Treated F	Control G	Treated I	Treated J	Control K

3.2.3 Results and discussion

Table 4 shows the pH and temperature data after passage through the WAM. There were no obvious changes to the wine. pH meters based on glass electrodes often return variable results, and in the author's experience the values are the same within meter error.

Table 4 pH and temperature of the wine after treatment with the flow-through WAM

	Treatment								
	High flow, 0.73 mL s^{-1}			Medium flow, 0.41 mL s^{-1}			Low flow, 0.14 mL s^{-1}		
	A	B	C	E	F	G	I	J	K
pH	3.24	3.24	3.27	3.28	3.22	3.23	3.16	3.15	3.23
°C	22	22	22	21	21	21	22	23	23

A typical UV difference spectrum is shown in Figure 21. Changes due to exposure to the electric field appeared to occur below about 350 nm. However, reproducibility was poor because replicate vials gave different responses. Thus, absorbances for E versus G were negative and for F versus G were positive; the parallel comparisons A and B versus C returned different curves although both negative; I and J were again different although both were positive.

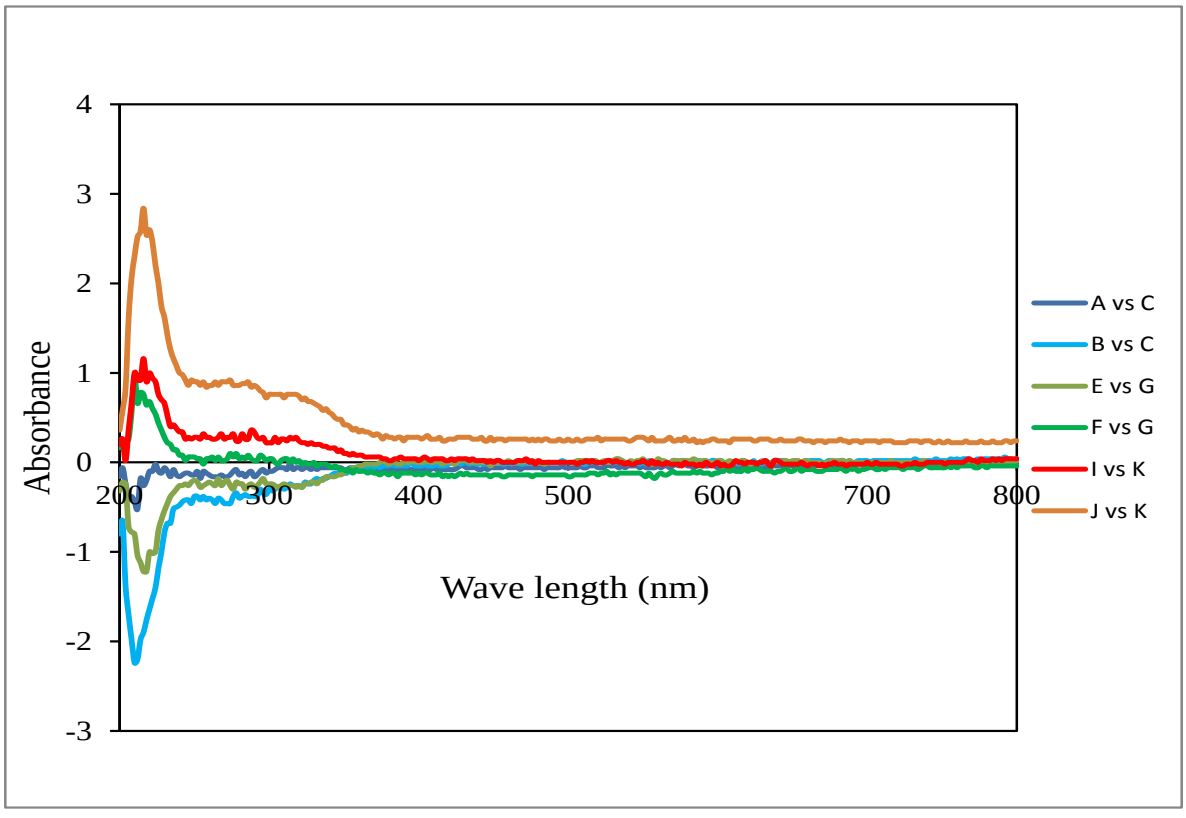


Figure 21 Absorbance difference of flow-through WAM treated wine and control wine

The repeat of these treatments was similarly tested for changes in absorbance summarised in

Figure 22, Figure 23, Figure 24 and Figure 25, except that there were two controls for each speed. pH and temperature were not recorded because the data in Table 5were clear.

Table 5 Basic treatments and dilution treatments for pilot study with white wine, all 1:20 with water

Treatments											
High flow (90%)				Medium flow (50%)				Low flow (20%)			
Treated		Control		Treated		Control		Treated		Control	
A	B	C	D	E	F	G	H	I	J	K	L

Figure 22 shows data from the repeated WAM treatment, generating six curves that repeated those in Figure 21(temporarily ignoring the three additional controls D, H and L). The curve colours are matched in both Figures. At first sight there appears to be a positive change in absorbance, peaking at around 7(for I versus K) in the Low flow treatment. Encouragingly, the repeat of the Low flow treatment (J) peaked around 6. A similar situation was observed for the two peaks from the Medium flow treatment (E and F), where the duplicates peaked at 2 and 5 respectively. The peaks from the High flow treatment (A and B) showed good reproducibility.

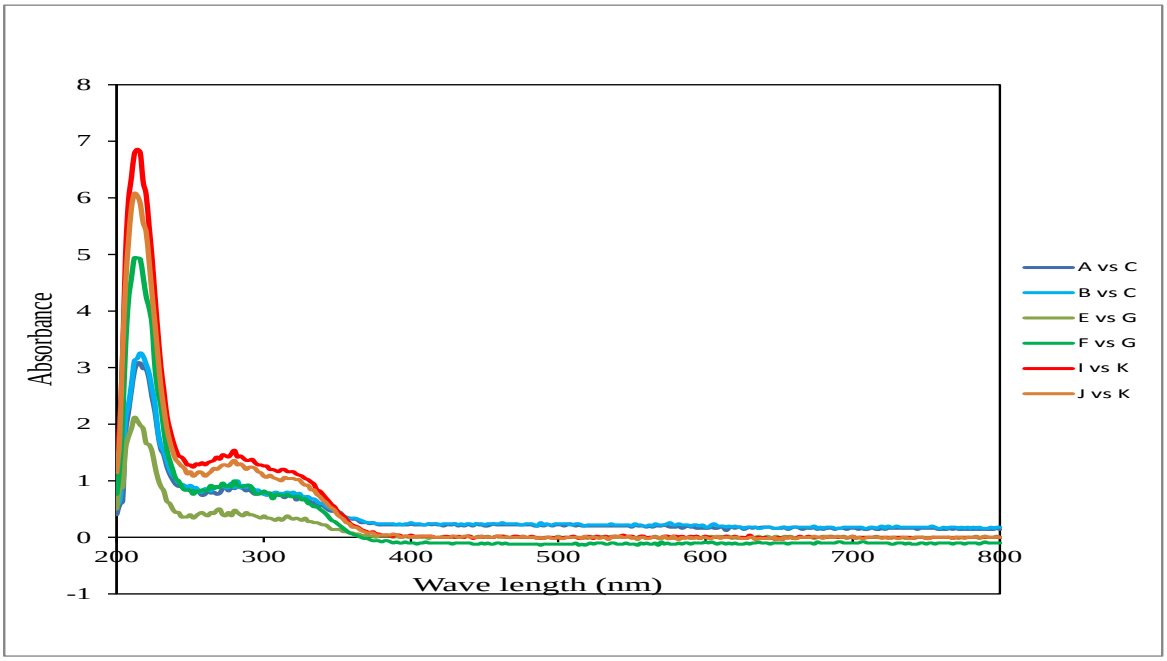


Figure 22 Absorbance difference of replicate for flow-through WAM treated wine and control wine

Figure 23 shows absorbance differences where the spectrophotometer was sequentially zeroed on

two duplicates (low flow WAM-treated in this case) and the differences were recorded within and between duplicates. Thus the graph compares I versus I, I versus J, and J versus J. The absorbance differences were minor and indicated that the wines were identical. A similar result was obtained for the equivalent medium flow comparisons (Figure 24).

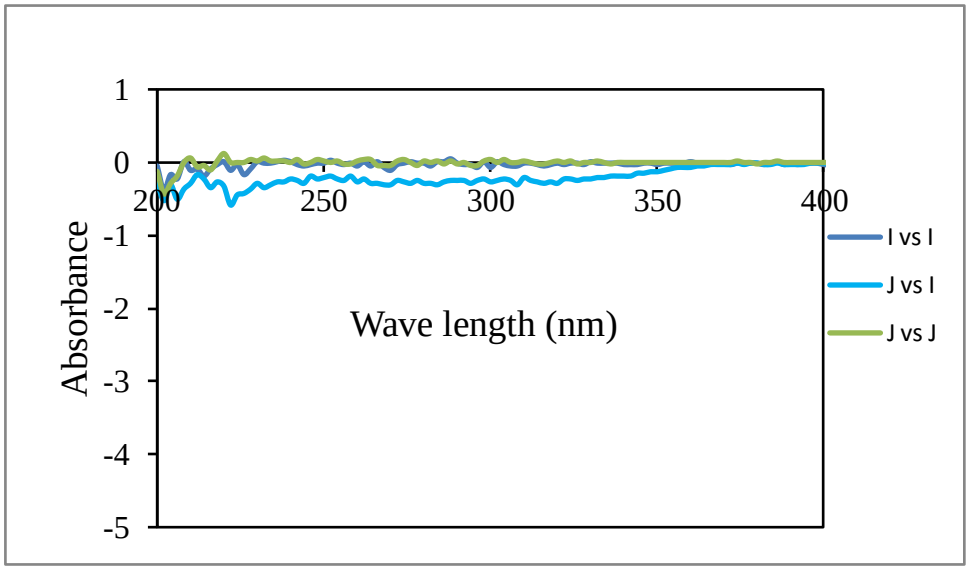


Figure 23 Absorbance differences within and between low flow WAM-treated wine duplicates

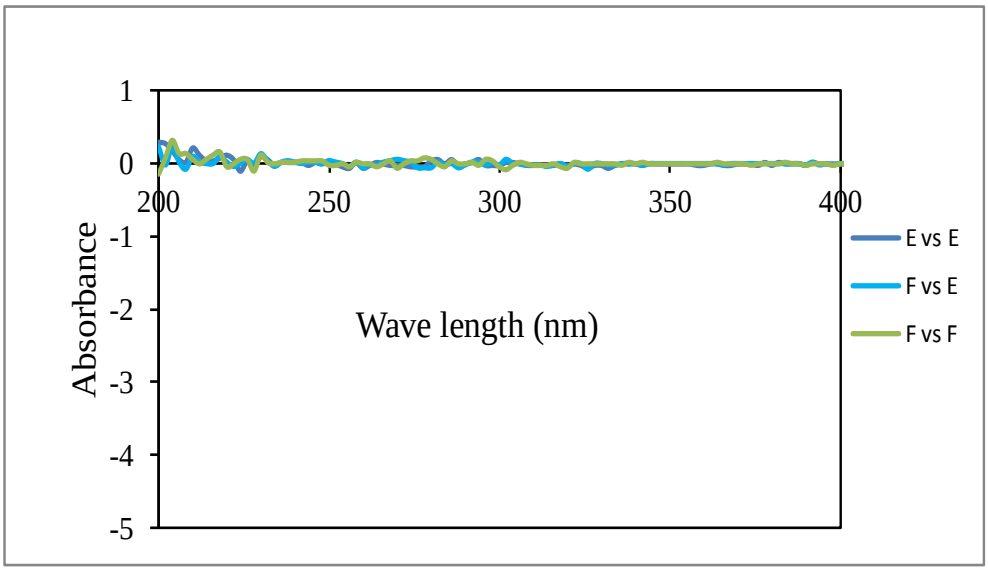


Figure 24 Absorbance differences within and between medium flow WAM-treated wine duplicates

However, the High flow rate data (Figure 25) strongly suggests that the absorbance differences are

artifactual; A versus A was very different. Repeats of this type of experiment gave similarly variable results (data not shown). It must be pointed out that in these comparisons a simple error was made. Consider I versus I in Figure 23. To generate the difference spectrum, the cuvette was simply refilled with the same diluted treatment I, but in the case of I versus J it was refilled with diluted J. Thus there is likely to be less variability due to dilution errors in I versus I than in I versus J. Ideally, a duplicate dilution of I should have been made in the comparison I versus I. This error means that like versus like comparisons are likely to be less variable than like versus unlike comparisons, and so it appears to be in Figure 25. If true, the data also suggest that errors in dilution are more likely to be the source of variability than electronic instability in the spectrophotometer.

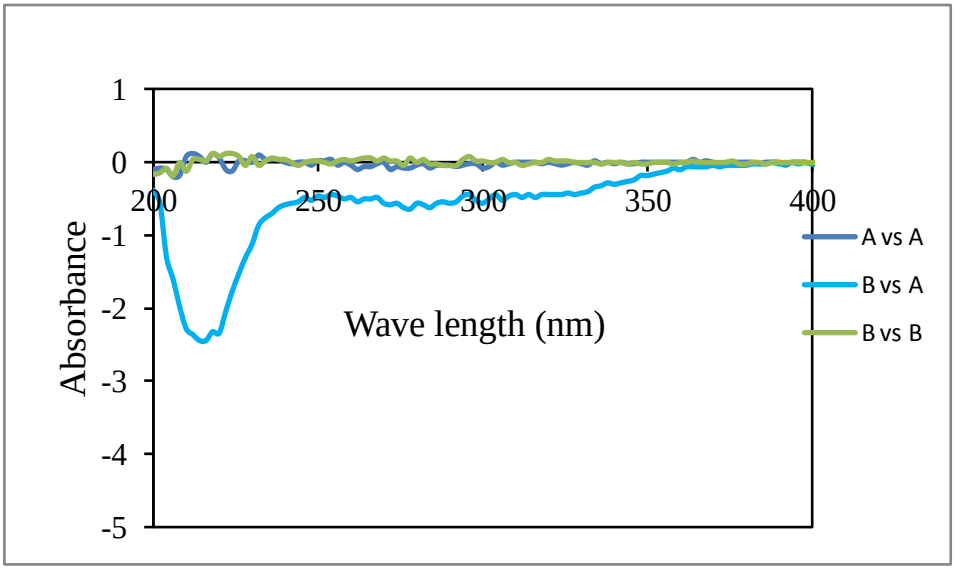


Figure 25 Absorbance differences within and between high flow WAM-treated wine duplicates

The idea that dilution was the cause of variability was further examined in repeats of this work, with the same 'error' in dilution.

Figure 26, Figure 27 and Figure 28 show that like versus unlike comparisons deviated more from the baseline than like-versus-like comparisons.

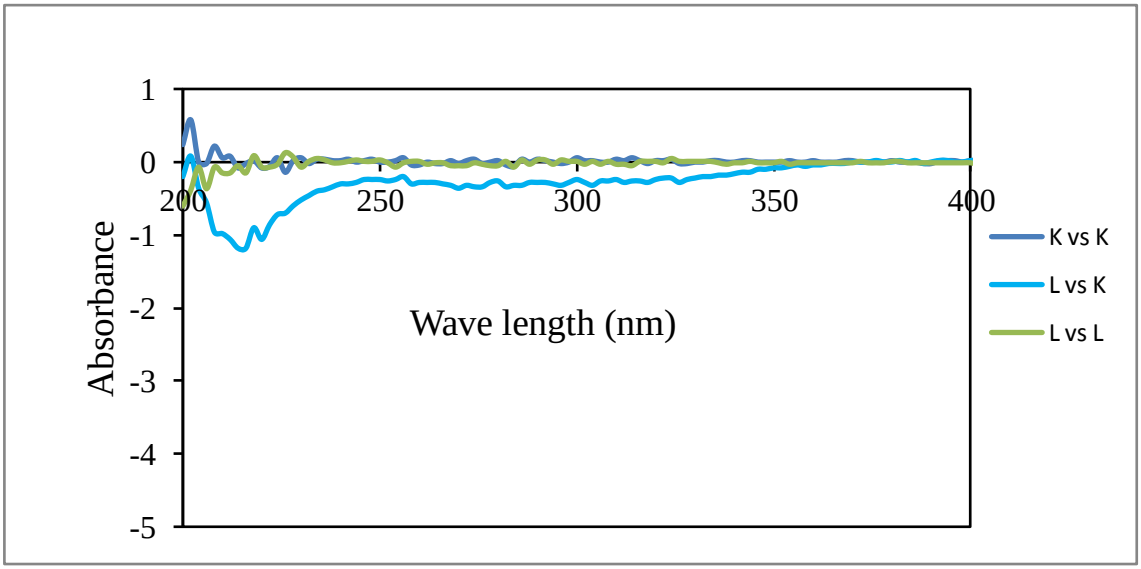


Figure 26 Absorbance differences within and between low flow WAM-treated control wine duplicates (repeat)

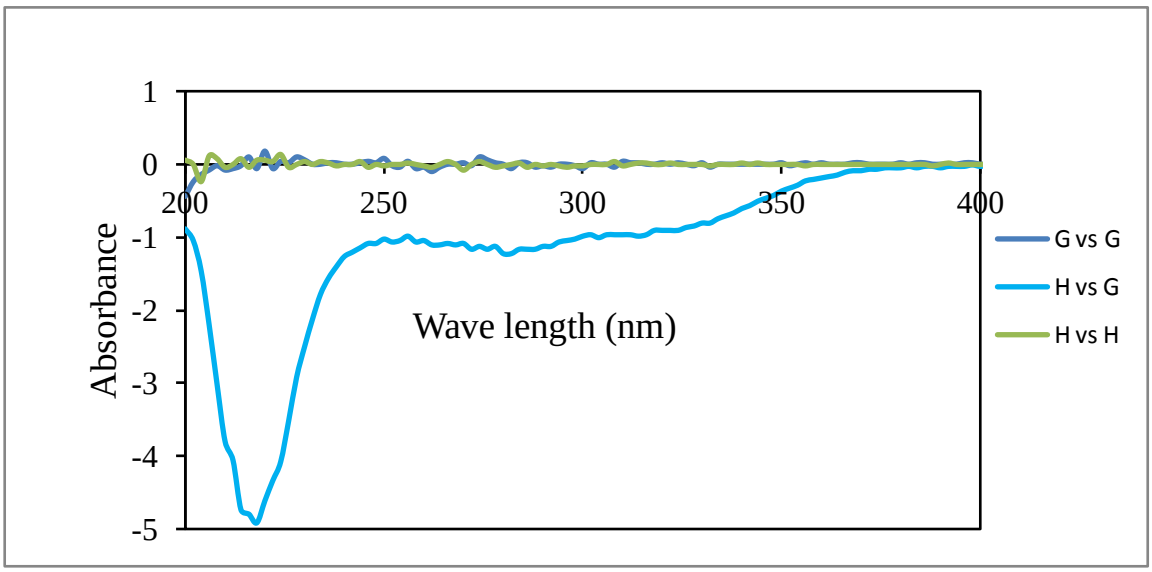


Figure 27 Absorbance differences within and between medium flow WAM-treated control wine duplicates (repeat)

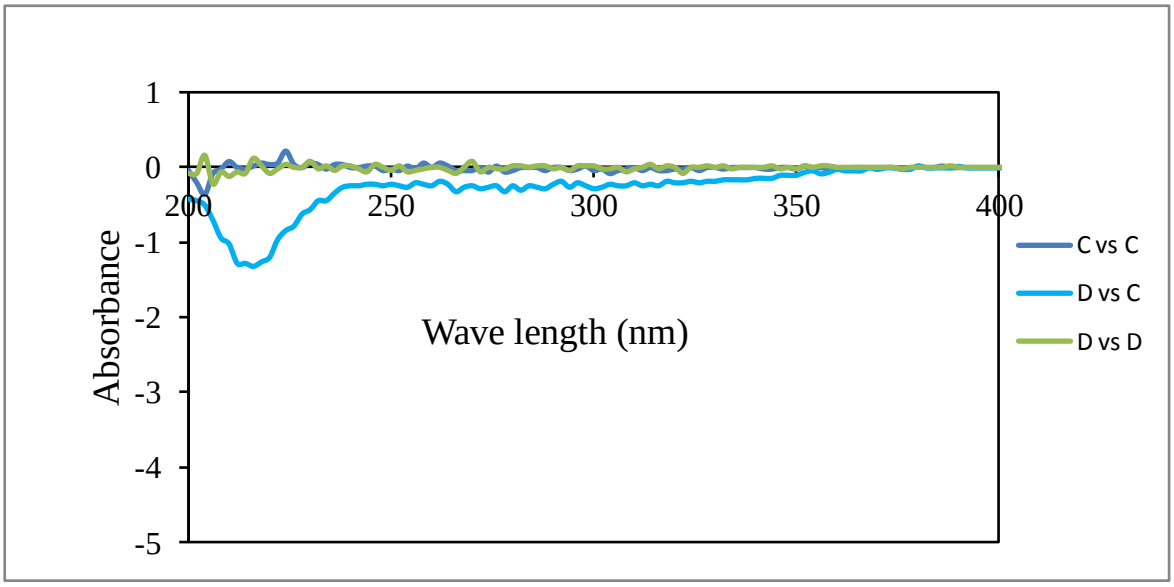


Figure 28 Absorbance differences within and between high flow WAM-treated control wine duplicates (repeat)

The conclusion of this work with white wine using the flow-through WAM, was that if there were changes caused by the electric field they had no measurable effect on compounds that absorbed in the visible and UV ranges. About this time the static WAM became available. It offered far more experimental control so the next set of experiments were done with it.

3.3 Wine treated by a static WAM

3.3.1 Introduction

With the failure to detect colour changes using the flow-through WAM, attention was directed to the more versatile static WAM. For reasons outlined in Chapter 2, a Sauvignon Blanc wine was chosen for this work. Moreover, it was the same wine used in the previous experimental section.

3.3.2 Methods

The frequency of wine aging machine (WAM) was set to 3046 Hz and the voltage to 501 root mean square volt per centimetre (Vrms cm^{-1}). The wine was poured into a 50mL beaker and then transferred into the 11mm x 150 mm test tubes. These test tubes were placed in the rack (Figure 13). The wine was treated by the electric field for 5, 10 and 30 min. The wine also distributed among several tubes in the same rack, but these were not exposed to the electric field. These were the controls. The WAM-treated and control wines were distributed among 2mL vials which were filled to over flowing so as to exclude air, and were stored at 4°C until required for use. The treatments are summarised in Table 6. There were three tubes of wine for each treatment. The tubes were located into the rack randomly in order to minimise any possible effect of position-in-rack on the wine.

Table 6 Treatments of white wine in the electric field of a static WAM

Position in the rack	1	2	3	4	5	6	7	8	9	10	11	12
Residence time (min)	5	30	0	10	5	30	0	10	30	0	10	5
Vials scanned	2	2	1	2	2	2	1	2	2	1	2	2

The wines were diluted 1:20 with deionised water, and the absorbances were recorded using the Ultrospec 2100 Pro UV/Vis spectrophotometer as described in Chapter 2. The pattern of scanning was that the two 5 min treatments in Position 1 were compared with the control in Position 3 etc. Thus six curves were generated for each exposure time.

3.3.3 Results and discussion

Figure 29, Figure 30 and Figure 31show the difference spectra for the three exposure times, between 200 and 400 nm. (Between 400 and 800 nm there were no differences, data not shown, and confirming the results in Figure 21. The results are variable particularly in the case of the 30 min exposure, where the absorbance difference at around 210 nm varied from 0.8 to –9. The fact that these were nominally identical replicates strongly suggests that the exposure had no effect.

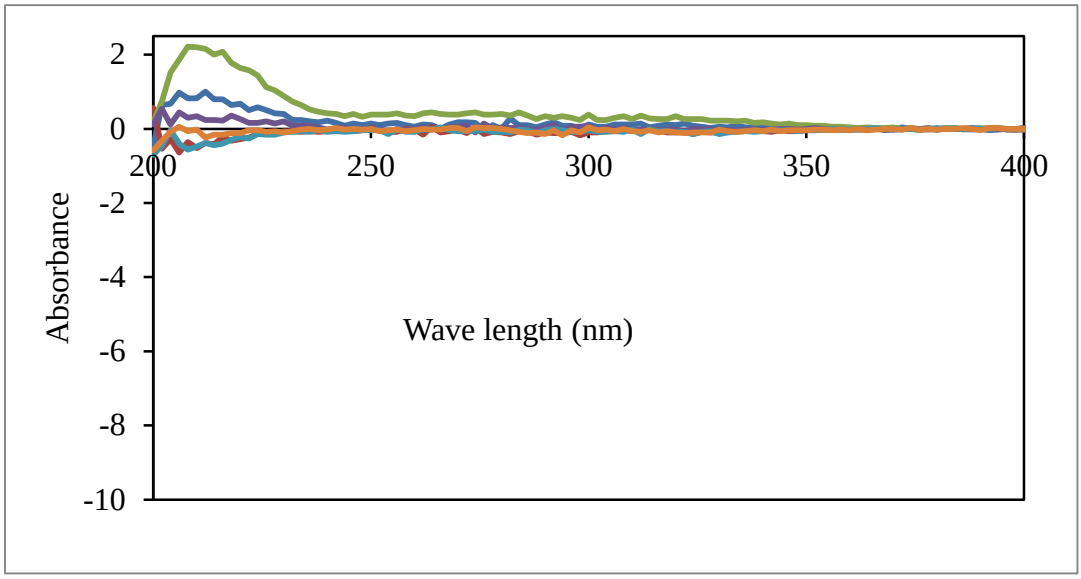


Figure 29 Absorbance difference of static WAM treated wine (5 minute) and its control.

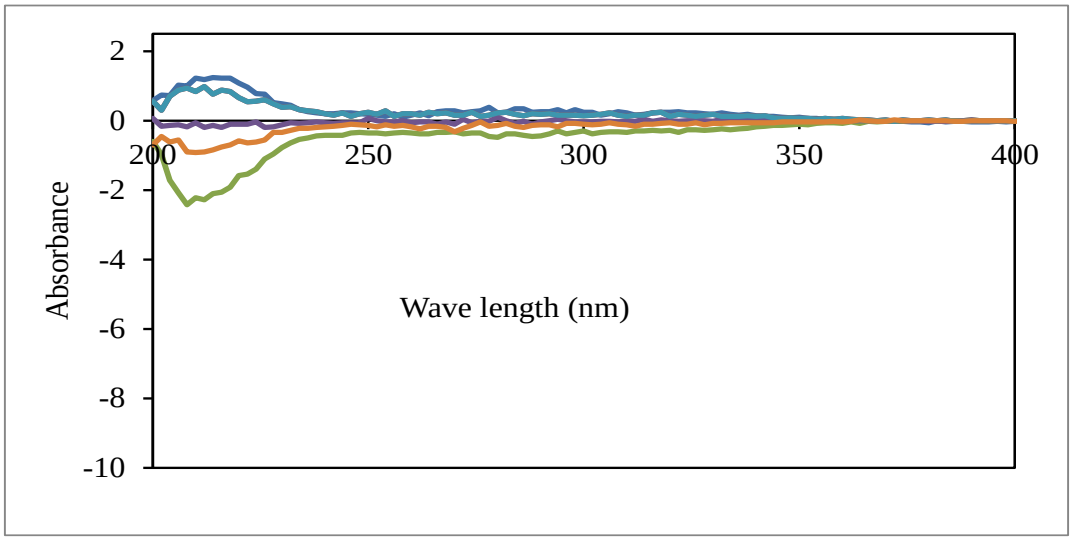


Figure 30 Absorbance difference of static WAM treated wine (10 minute) and its control.

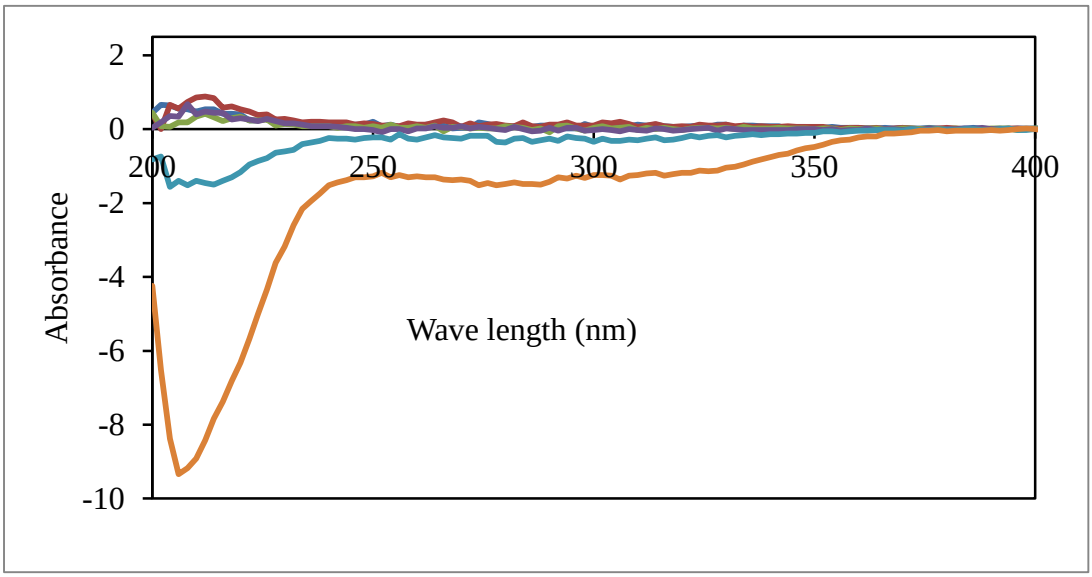


Figure 31 Absorbance difference of static WAM treated wine (30 minute) and its control.

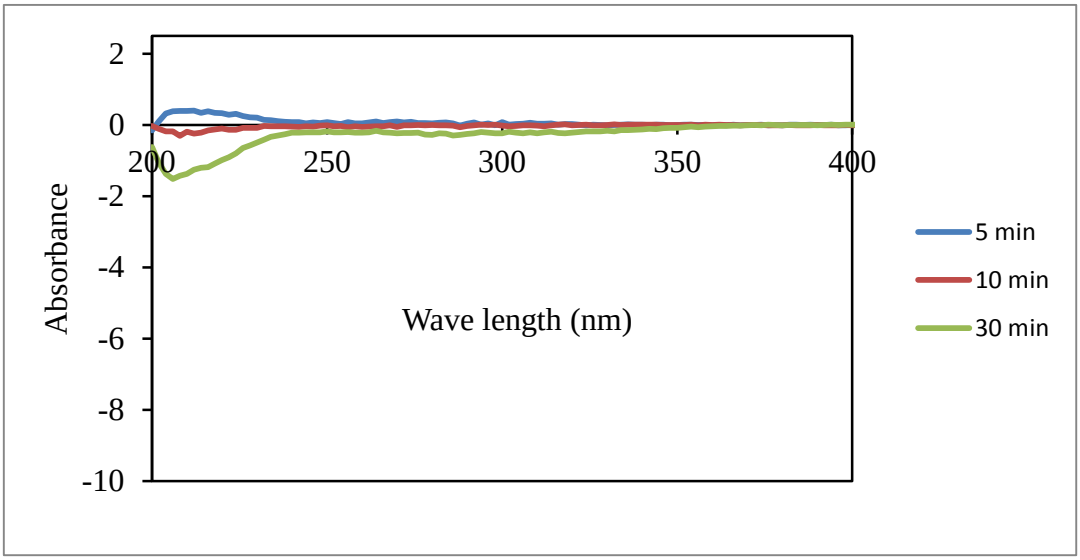


Figure 32 The mean UV-Vis spectrum of static WAM treated wine versus controls for three exposure times.

Figure 32 shows the mean of the six curves for each exposure. Error bars have not been included for clarity, because these would merge as a cloud. It is concluded that the treatments at 3046 Hz and 598 Vrms cm^{-1} for varying times had no effect on colour, suggesting that there was no changes to light absorbing compounds by these treatments. It cannot be concluded, however, that there was

no effect at all, as might be judged by sensory evaluation for example.

3.4 Wine treated by the static WAM with different frequencies and strengths of electric field

3.4.1 Introduction

The previous experiment showed that at in a given field strength and frequency, length of exposure (0 to 30 min) had no effect on colour. In this experiment, the same white wine was treated by the static WAM with different frequency and strength of electric field combinations.

3.4.2 Methods

The wine was treated by the static WAM for five minutes. The WAM settings are shown in Table 7. There were three chosen frequencies, each frequency was set with four different strengths of electric field for wine treatment. Three controls (completely unexposed to a field) were used, one for each frequency. All aliquots were diluted 1:20. Duplicate dilution was made for each static WAM treated wine or control. The curves represent the means of two scans.

Corrected for dilution, the peak values all lay between 0.7 and -3.5. To show these data it would appear logical to scale all the graphs between, say 1 and -4.0. However, to make these data comparable to the previous results with the static WAM it was decided to scale the graphs between 3 and -10.

Table 7	Frequency and strength of electric field in the static WAM, using Sauvignon Blanc

Frequency (Hz)	Strength of electric field (Vrms cm^{-1})
3046	299
	598
	906
	1205
3553	299
	598
	906
	1205
4145	299
	598
	906
	1205

3.4.3 Results and discussion

Figure 33, Figure 34 and Figure 35 show the absorbance difference of static WAM treated wine set to different frequencies with different strengths of electric field compared to Control wine.

In Figure 33, at 210 nm, 1205 Vrms cm^{-1} showed the lowest absorbance difference (-0.7) while 598 Vrms cm^{-1} showed the highest absorbance difference (0.7). However in Figure 35, at 210 nm 1205 Vrms cm^{-1} showed the highest absorbance difference (0.5) and 299 Vrms cm^{-1} showed the lowest absorbance difference (-0.5). These results indicate that the absorbance difference does not show any pattern as the strength of electric field increased. The absorbance difference did not show any trend as the frequency of the electric field increased.

In Figure 33, wine treated at 299 Vrms cm-1 and 3046 Hz showed an absorbance difference at 210 nm of about 0.2. With the same strength of electric field, the absorbance difference at 210 nm showed similar value when frequency of electric field was 3553 Hz (Figure 34) but a different value (-0.5) when the frequency was increased to 4145 Hz (Figure 35). Similarly, when wine was treated at 598 Vrms cm-1 and 3046 Hz (Figure 33), the absorbance difference at 210 nm was 0.7. With the same field strength, as the frequency increased to 3553 Hz and 4145 Hz, the absorbance

differences at 210 nm were both about 0.2 (Figure 34and Figure 35).Thus, there was no pattern to these curves. Moreover, where the controls were compared with one another (Figure 36), one difference that should have been zero at 200 nm was -3.5.

Clearly the differences were artifactual, and may be due to dilution errors and/or electronic noise in the spectrophotometer. But it is also important to realise that lack of a colour changes does not necessarily mean there was no effect. Attention was then directed at sensory evaluation.

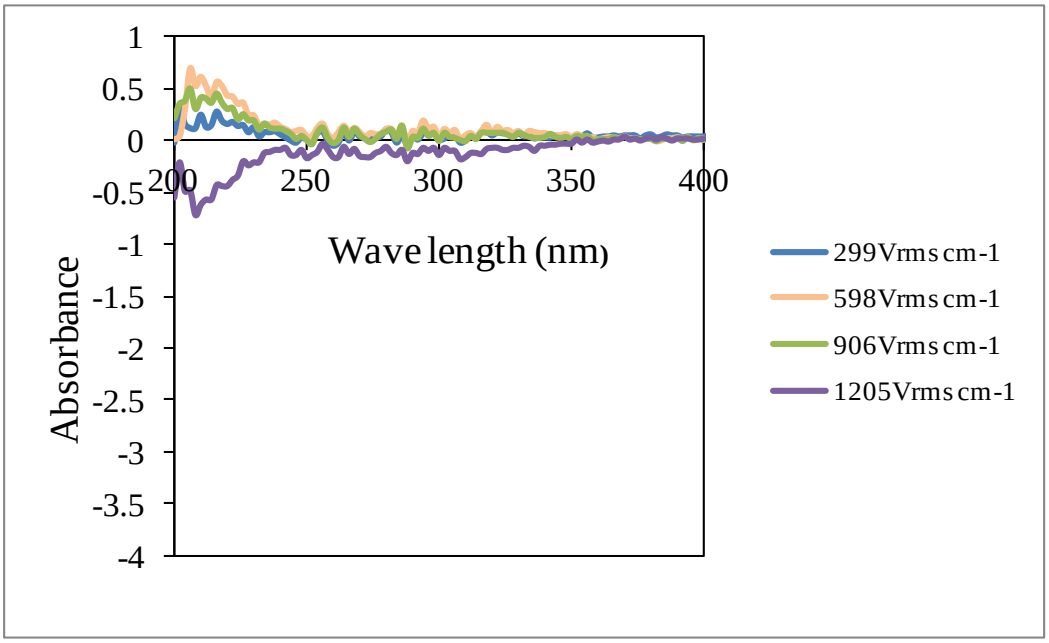


Figure 33 Absorbance difference of static WAM treated wine set to 3046 Hz compared to the control

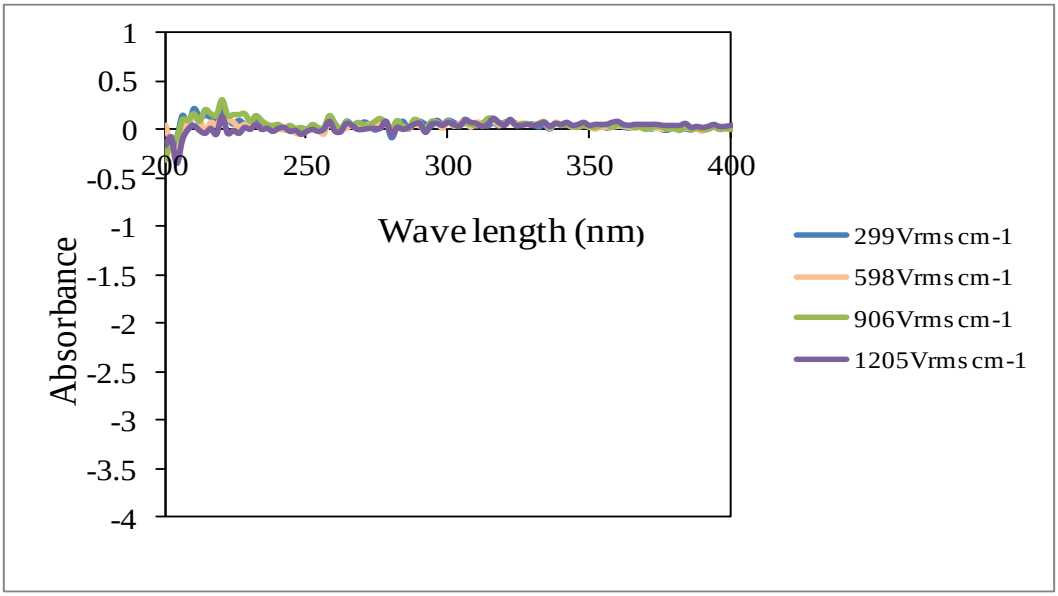


Figure 34 Absorbance difference of static WAM treated wine set to 3553 Hz compared to the control.

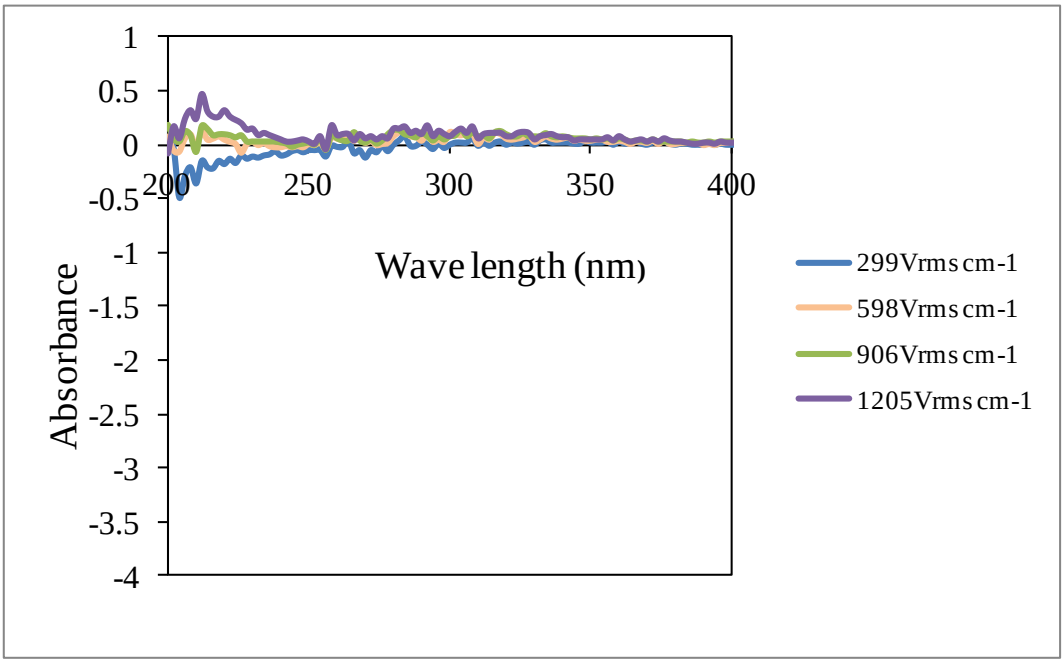


Figure 35 Absorbance difference of static WAM treated wine set to 4145 Hz compared to the control.

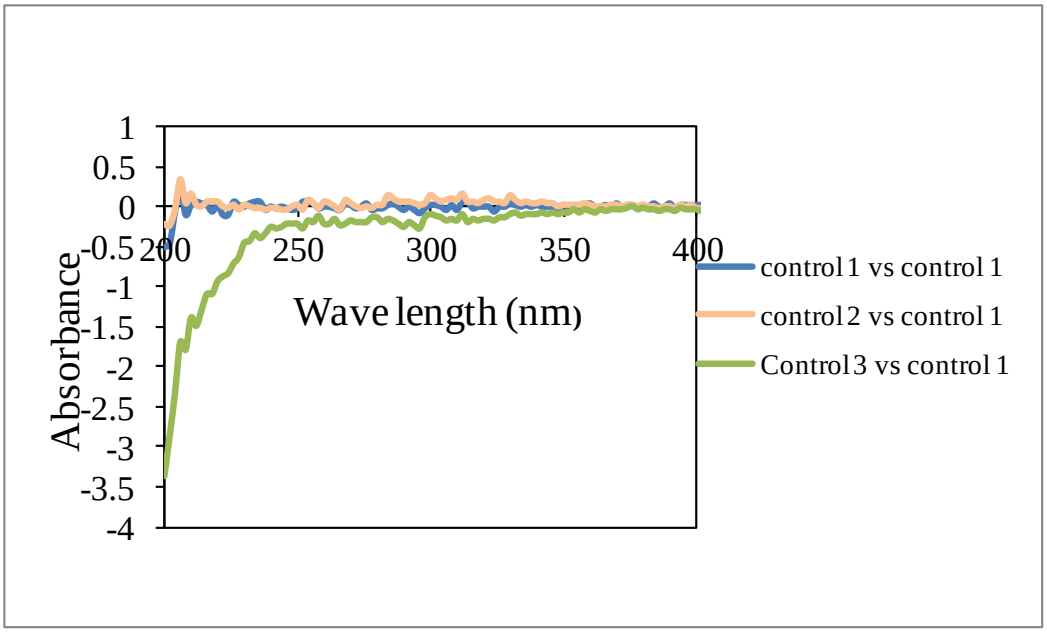


Figure 36 Absorbance difference of controls compared with one another.

3.5 Sensory analysis of wine treated in a static WAM

3.5.1 Introduction

The previous experiments showed that static WAM did not have any effect on wine colour, but as noted this does not necessarily mean it has no effect. Sensory tests were conducted to find out the effect of static WAM on wine smell, usually called aroma in the case of wine.

3.5.2 Methods

First, an Astrolabe Marlborough Sauvignon Blanc 2010 was treated by the static WAM at a frequency of 3046Hz and the strength of electric field is 598 $Vrmscm^{-1}$ for 10 min. There was no particular reason for using these conditions. The wines were evaluated for difference in smell with a panel comprising 34 AUT students and staff. Many of the panellists were wine drinkers, but were not recognised connoisseurs. The wines were evaluated by discriminative analysis using the triangle test.

Subsequently, two bottles of EskValley Merlot Cabernet Sauvignon Malbec 2009 were each treated by the static WAM under the conditions as above. One was freshly opened and the other had been open for three days exposed to air at room temperature, but sealed under the screw cap. The wines were similarly evaluated in two separate discrimination trials.

3.5.3 Results and discussion

The sensory results (Table 8) show that there were no significant smell differences due to exposure to the electric field for any of the three wines.

Table 8 Discrimination test for smell between treated and untreated wines

	Astrolabe	EskValley (freshly opened)	EskValley (previously opened)
Number of panellists	34	14	24
Number correct	15	3	9
Significance	Not signif.	Not signif.	Not signif.

On the face of it there was no change in these wines at all. This was further explored by HPLC mass spectrometry, as described in the next section.

3.6 White wine with the flow-through WAM version 2

3.6.1 Introduction

The flow-through WAM was sometimes not available for research at AUT because of the demands of other users in Hamilton. That was one reason for using the static WAM, but as shown previously the results were all negative. Claims for the WAM technology have all been based on the flow-through design and it was thought that the motion of wine in the electric field could be important for an effect. Therefore, when the opportunity arose to do further work with the flow-through WAM, it was taken.

Unfortunately the flow-through WAM failed, and the electronics had to be rebuilt by Mr Brett Holden of AUT (Chapter 2). This WAM is designated flow-through WAM version 2, here tested with the sole white wine under study. There were also three other changes described in Methods.

3.6.2 Methods

In assessing UV absorbance, there were three departures from previous methods. The most important of these was the switch to the newer Ultraspec 7000 UV/Visible spectrophotometer. This is a double beam instrument and where wine was analysed water was placed in one cuvette and diluted wine in the other. Second, the diluent and water used in the reference cuvette was changed from standard laboratory deionised water to Milli Q. The properties of these two water is discussed in the following text.

The suitability of using Milli Q water was determined by conducting triplicate scans of the two waters (changing the water for each scan) in a single quartz cuvette between 200 and 800 nm. The reference beam passed through air.

The white wine was the Astrolabe 2010 sauvignon blanc.Two bottles were pooled and wine was poured into the reservoir of the (flow-through) WAM 2. The peristaltic pump was set to 900, which generated a flow of 0.476 mLs^{-1}.There were two passes, one with the electric field off and one on.The field was set to 3000 Hz and 600 Vrms cm^{-1}. This field-treated wine was designated WAM2 wine to be compared with the control wine (field off).The wines were immediately distributed among overfilled vials. For WAM and control, five of these vials were each diluted three times with Milli Q water (1:20) and scanned between 200 and 400 nm. The reference cuvette

contained Milli Q water, rather than with diluted control wine, and the test cuvette contained the replicated diluted wines under study. In this configuration the absolute spectrum was obtained for each wine.

The mean for each vial was the basis for statistical analysis. In the wavelength range chosen (230 to 350 nm), each of the 10 values (five from each treatment) was subtracted from the mean of those 10 values at each wavelength. Next, equal variance was assumed and this was plausible because the curves were similar. The null hypothesis was that the differences from the mean over the wavelength range were equal, and this was tested by a one way t Test.

Sensory test was done as described in Chapter 2 (results presented along with sensory results for red wine.)

3.6.3 Results and discussion

Figure 37 shows the UV spectra of Milli Q water and deionised water both compared to air. Deionised water showed highest absorbance of 0.56 at 200 nm. It peaked about 0.25 at 220 nm and the absorbance decreased to 0.04 at 280 nm. The absorbance of Milli Q water and deionised water were the same from 280 to 800 nm. For Milli Q water, the highest absorbance was 0.09 at 200 nm, the absorbance decreased to 0.04 at 280 nm and maintained the same absorbance as deionised water as the wavelength increased. The higher absorbance of deionised water in 200 to 280 nm compared with Milli Q water suggested that Milli Q water is better diluent than distilled water. It was used in all subsequent experiments.

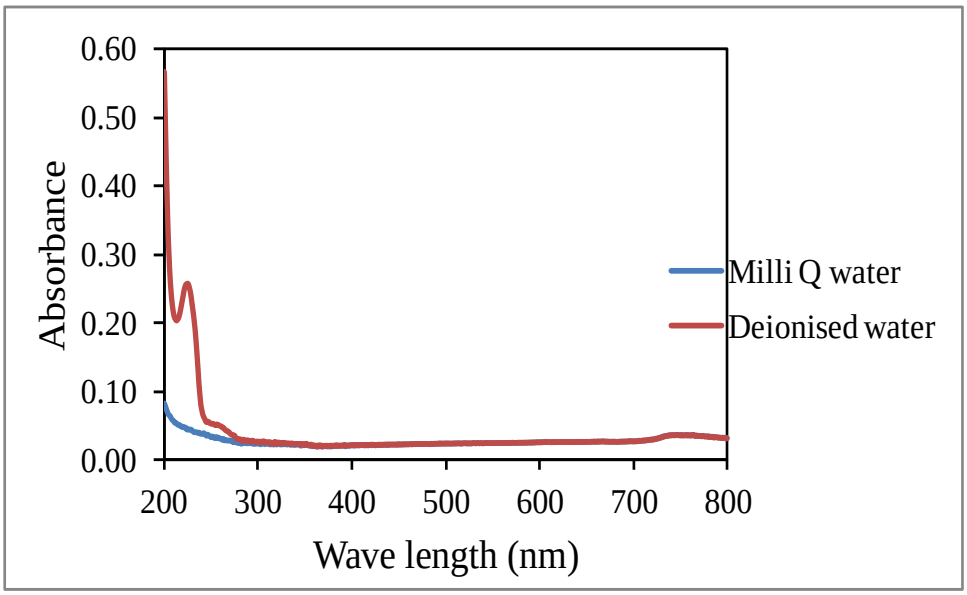


Figure 37 UV spectra of Milli Q water and deionised water compared with air

Figure 38 shows dilution corrected UV spectra of flow-through WAM2 treated and control wine comapred with (Milli Q) water. There was anapparent absorbance difference between WAM2wine and control wine in the wavelength range of 240 to 340 nm. WAM2 wine showed a higher absorbance than control wine at least in that range, while the Milli Q water compared with Milli Q water showed no apparent difference. The null hypothesis was rejected (P = 0.00498). Thus it was concluded that the curves were significantly different between 230 and 350 nm.

This was the first significant difference shown in this study. It is important to realise however that significance is not the same as importance in an organoleptic sense. Thus, if the significant absorbance difference shown in Figure 38 translated to a difference in organoleptic perception, then it would be important. If not, the significant absorbance difference would be unimportant. Equally it would be important in a chemical sense in that what is changing in the wine and why.

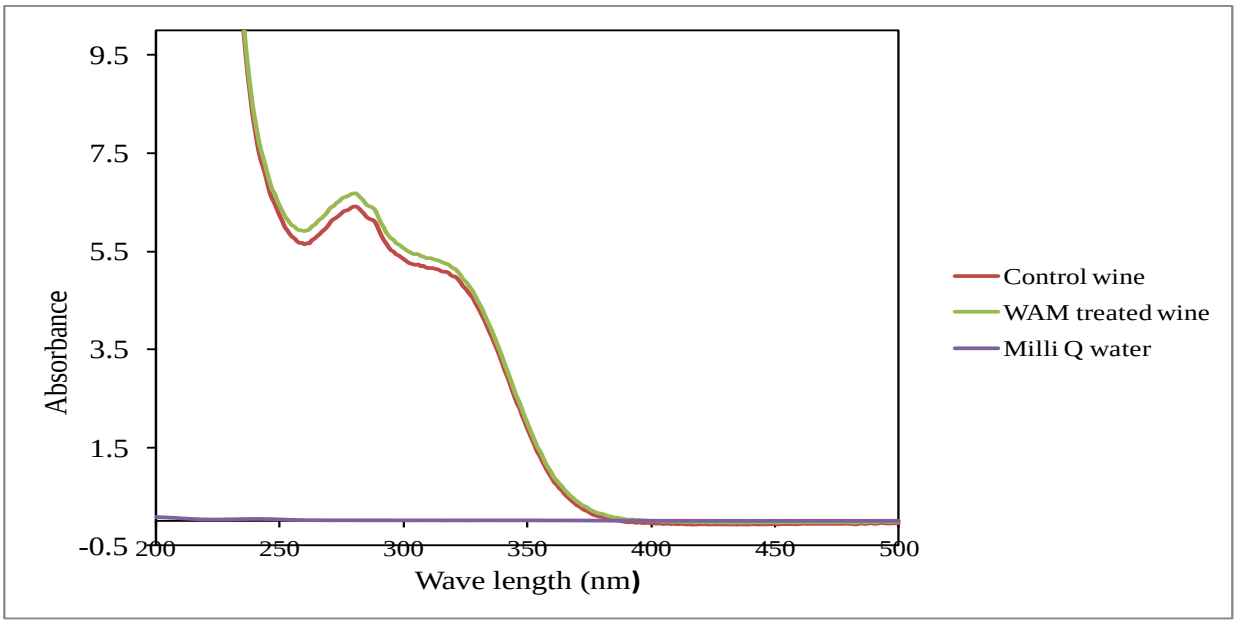


Figure 38 UV-Vis Spectra of white wines

3.7 Red wine with the flow-through WAM version 2

3.7.1 Introduction

The previous section showed that there was significant absorbance difference in the wavelength range of 230 to 350 nm between white wine treated inWAM2 and control wine. Talele et al. (2013) that a flow-through WAM had a significant effect on red wine. Therefore, the work with white wine above was repeated with red wines.

3.7.2 Methods

The red wines used were Hardy's Cabernet Merlot, non-vintage (Australia), Banrock Station Merlot Cabernet 2011 (Australia), and Mill Road Pinot Noir 2011(New Zealand). Work with Mill Road Pinot Noir was tested in three ways, while the other wines were tested according to a single method.

The methods used were closely similar as described for the white wine with WAM2, and are shown in Table 4. The peristaltic pump was used to control the flow rate for Hardy sand Banrock Station, and the electric field was set to 3000 Hz and 600 Vrms cm^{-1}.The maximum flow rate achievable with the peristaltic pump was 0.476 mL s^{-1}, and as discussed earlier, it was thought

possible that a high flow rate might be important to induce an effect. Therefore, a method was designed for increasing the flow rate of wine in flow-through WAM2. Rather than 'push' the wine through the flow-through WAM2, as was done for Hardy's and Banrock Station, a vacuum was used to 'pull' the wine through. The design for this was described in Chapter 2. The vacuum method at least halved the retention time. Although the vacuum method was effect in achieving a high flow rate, it was relatively uncontrollable due to variable resistance as wine completed passage through the tubing. Another variation to the usual technique was that two of the three Mill Road wines were cycled through flow-through WAM2 three times, in one of which the electric field which was set to 3000 Hz and 1200 Vrms cm^{-1}.

At this point of the project, it was realised that the work to date lacked a simple control beyond the obvious control of wine passing through a WAM with the electric field off. This second control was wine that had not passed through WAM2 at all, and was called initial wine. The initial control was applied to work with Banrock Station and Mill Road I (Table 9).

The dilution for red wine was 1: 100 with Milli Q water. The absorbance of all the diluted wine were determined by Ultraspec 7000 UV/Visible spectrophotometer by setting Milli Q water as baseline.

Table 9 Treatment of red wine with WAM2

	Hardy's	Banrock Station	Mill Road I	Mill Road II	Mill Road III
Frequency (Hz)	3000	3000	3000	3000	3000
Strength of electric field (V rms cm^{-1})	600	600	600	600	1200
Flow method	Peristaltic	Peristaltic	Vacuum	Vacuum	Vacuum
Number of cycles through WAM2	1	1	1	3	3
Approx. retention time (min)	7	7	3	9	9
Initial control[1] applied?	No	Yes	Yes	No	No

[1] An initial control is where the wine was not passed through the WAM2 at all.

Sensory tests were done as described in Chapter 2 and Table 10.

3.7.3 Results and discussion

All the absorbance results obtained from different brands of red wine were very similar.

Figure 39 shows the UV spectra of diluted WAM treated wine and control wine. The absorbance difference of Milli Q water versus Milli Q water is zero which indicats that there was minor error from the replication and instrument. The control wine and re-build flow-through WAM treated wine overlapped each other perfectly. The similar results were shown for Banrock Station Merlot Cabernet (Figure 40) Mill Roads (Figure 41, Figure 42 and Figure 43). It indicates that the redesigned flow-through WAM did not have any effect on colour.

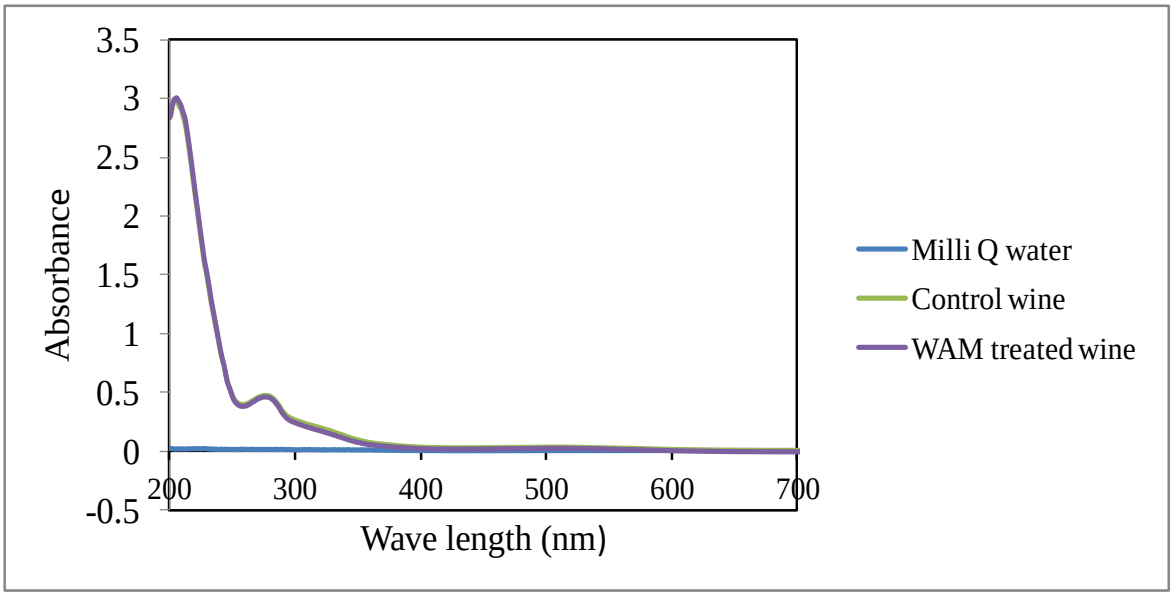


Figure 39 UV-Vis Spectra of Hardy's Cabernet Merlot

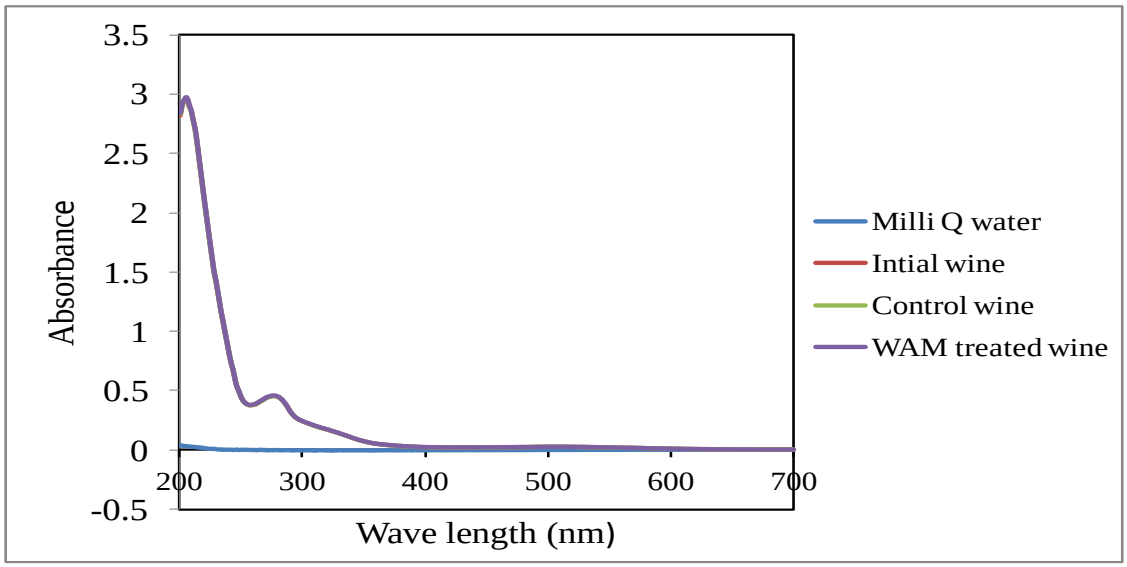


Figure 40 UV-Vis spectra of Banrock Station Merlot Cabernet

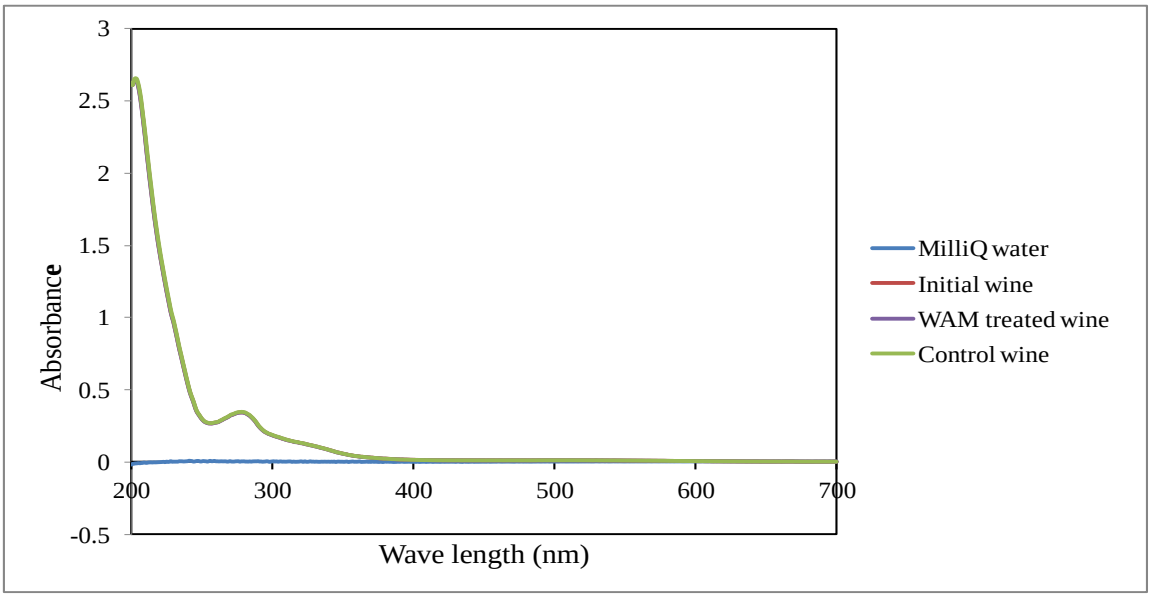


Figure 41 UV-Vis spectra of Mill Road Pinot Noir (I)

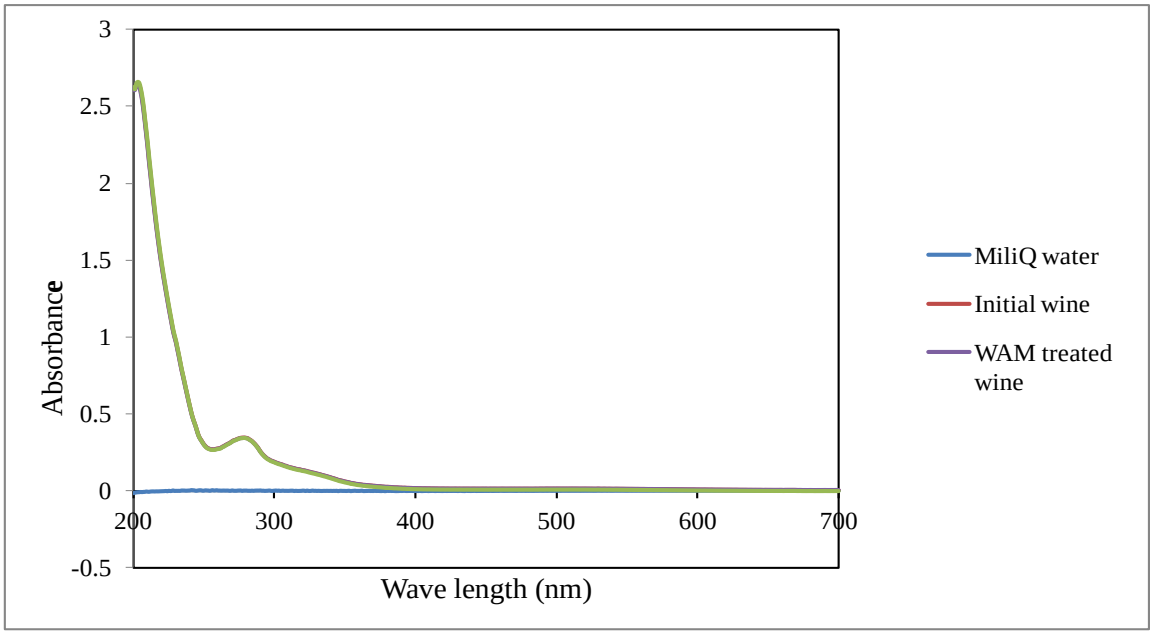


Figure 42 UV-Vis spectra of Mill Road Pinot Noir (II)

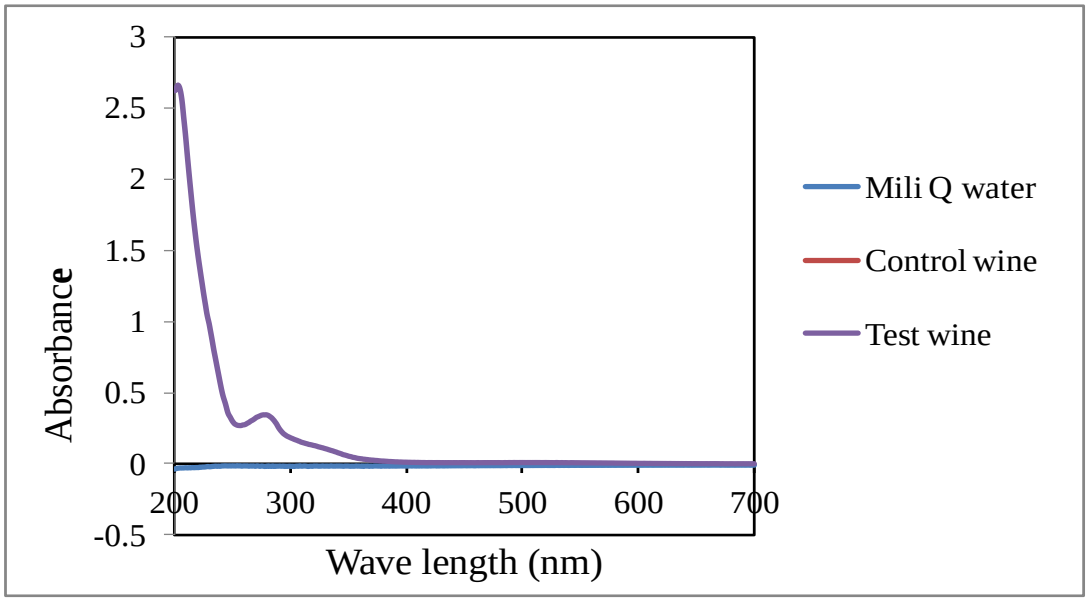


Figure 43 UV-Vis spectra of Mill Road Pinot Noir (III)

Results of sensory tests (Table 10) show that there was no significant aroma difference between the flow-through WAM 2 treated wine and control wine. It concluded that flow through WAM 2 did not have any effect on the aroma of wine.

Table 10 Sensory result for the flow-through WAM2 wines

Types of the wine	Sauvignon Blanc	Hardy's	Banrock Station	Mill Road I	Mill Road II	Mill Road III
Total number of participants	34	24	29	18	28	30
Number of correct responses	15	7	13	5	7	5
Significance	Not signif.	Not signif.	Not signif.	Not signif.	Not signif.	Not signif.

3.8 Liquid chromatographic analysis

3.8.1 Introduction

The static WAM trial in the previous section did not show any effect on aroma as determined by a discrimination test. Although it was tempting to conclude that the WAM had absolutely no effect on the wine, it was possible to analyse the wine by liquid chromatography coupled with mass spectrometric detection. The equipment used was a ultra (high) pressure liquid chromatograph with time of flight spectrometric detection, equipment not available at AUT at the time.

3.8.2 Methods

The Astrolabe white wine was treated by static WAM set to 3046 Hz and 598 Vrms cm^{-1} (Test 1), 3046 Hz and 1205 Vrms cm^{-1} (Test 2). Exposure was for three minutes for each. The Control wine was also poured into the exposure tubes but was not subjected to the WAM. The wine was poured into overfilled vials (described earlier) that were were immediately sealed without any air bubbles. They were refrigerated for two days, and later held at ambient temperature for a day during transit, followed by refrigerated storage in Hamilton.

Two-micro litre aliquots from different vials were auto-injected into a Zorbax Eclipse Plus C18 reverse phase column (100 mm x 2 mm, 1.8 μm particle size) fitted to an Agilent 1290 Infinity ultra performance liquid chromatograph (UPLC) (Figure 44). The mobile phase was based on a constant 0.1% (v/v) formic acid with a gradient over 19 minutes of acetonitrile from 2.5 to 100 % (v/v) as shown in Table 11. The flow rate was 0.4 mL min^{-1}. The post-run equilibrium time with 2.5% acetonitrile was 2.5 minutes. Each treatment had six replicate injections. The mass spectrometer recorded data in both negative mode and positive modes. The detector was the Agilent 6550 qTOF (time-of-flight) covering the range of 50 to 1700 mu in low mass resolution mode at 20,000 mass resolution, with high sensitivity. The temperature of the column was 40ºC.

Figure 44 An Agilent 1290 Infinity UPLC - MS

Table 11 Mobile phase gradient program of UPLC for wine

Time (min)	Formic acid (0.1%) in water (% v/v)	Acetonitrile in 0.1% formic acid (% v/v)
0	97.5	2.5
1	97.5	2.5
19	0	100
19.5	0	100
20	97.5	2.5
22.5	97.5	2.5

The molecular feature extraction algorithm was the MassHunter Qual software that was part of the analytical system.

The program R was used to select the common compounds that occur in all replicates, under the guidance of Ping Gao, a postgraduate colleague at AUT, who is skilled in the use of this program. The m/z values were run into three decimal places and retention times were run into two decimal

places. The R selected the compounds that have the same rounded m/z value and same rounded retention times. The selected compounds that exist in all replicates were chosen.

3.8.3 Results and discussion

During ionisation, a molecule with a molecular weight of M is protonated to create a positive ion $(M+H)^+$ in positive mode.

In contrast, when the molecule is ionised in negative mode, a molecule is deprotonated to create a negative ion $(M-H)^-$(Ashcroft, 2013). The positive mode ionization mode is used when the functional group of the molecule accepts protons easily, and vice versa. Whatever the mode, the molecular ion is subsequently fragmented into smaller ions. The mass to charge ration (m/z) of fragments provides information about the structure of the molecule. The chromatograph of one replicate in positive and negative mode are shown in Figure 45and Figure 46.

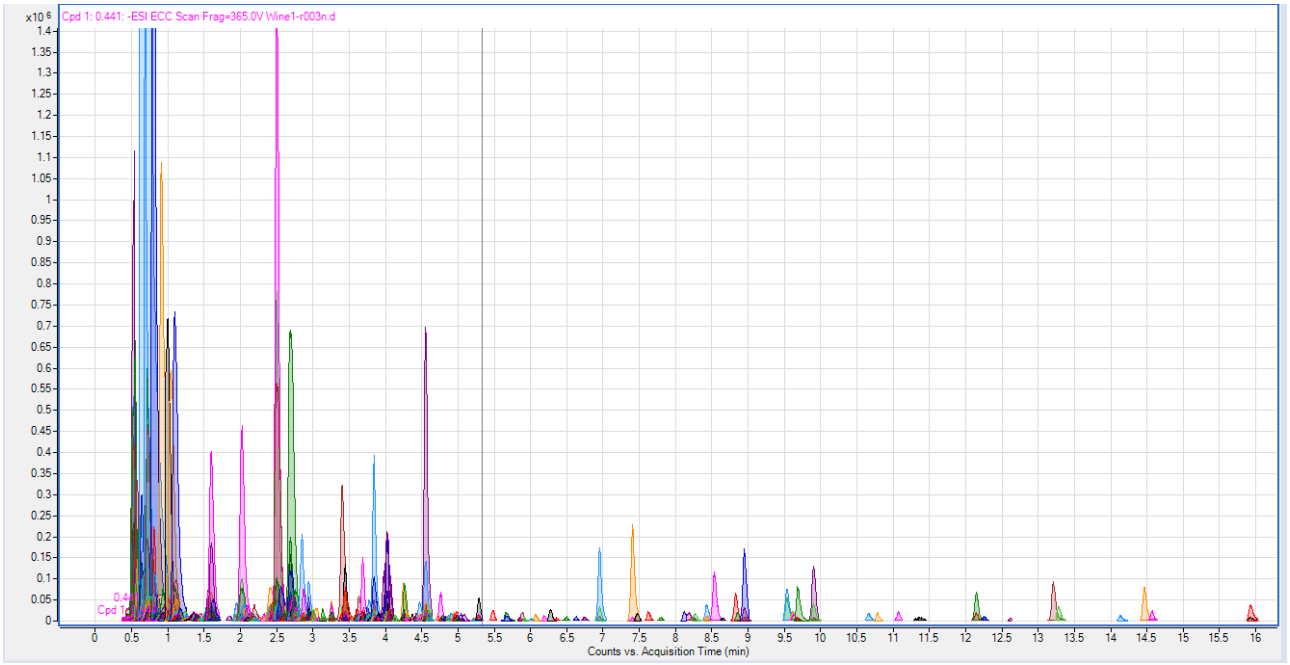


Figure 45 Test 1 analysed in negative mode of UPLC-MS

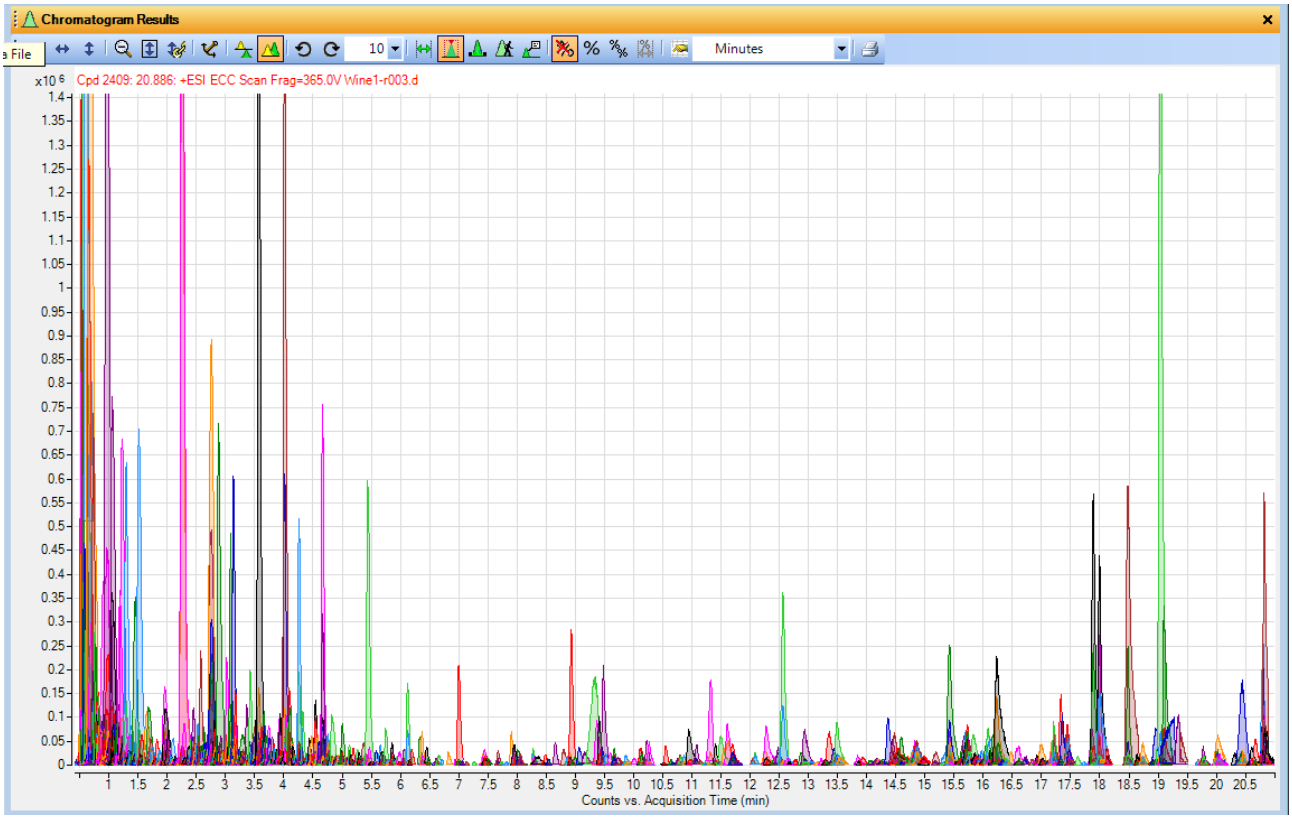


Figure 46 Test 1 analysed in positive mode of UPLC-MS

Eight hundred plus compounds were found in wine in the negative mode, and 1800 plus compounds in the positive mode (Table 12 and Table 13). Negative mode data were available for only three of the six replicates because of uncertainties about data integrity for those three, but only those three. In analysing these data it was vital to align the eluted compounds according to their m/z values and retention times. This was done by using R software briefly described in Methods above.

Table 12 Number of compounds detected in wines in negative ionization mode

Replication	Control wine	Test 1	Test 2
1	795	844	836
2	802	797	830
3	814	827	848
4	Not available	799	832
5	Not available	848	832
6	Not available	823	860
Mean	804	823	840

This in turn allowed the selection of compounds that were common to all replicates for each mode. The rationale was that the effect of the field – if any – had to be common to each replicate in each treatment. Thus in positive mode for example, if a peak was missing or below the limits of detection in just one of the 18 replicates, that compound did not make the shortlist. According to this criterion, seven compounds of 1800 plus were shortlisted in positive mode, and 50 in negative mode (Table 14 and Table 15). Thus the negative mode produced more replicable data the positive mode, so subsequent analysis focused on negative mode.

Table 13 Number of compounds detected in wines in positive ionization mode

Replication	Control wine	Test 1	Test 2
1	1722	1972	2003
2	NA	1956	1916
3	1888	1907	1923
4	1858	1900	1948
5	1912	1945	1926
6	1955	1967	1901
Average	1867	1941	1936

Table 14 Common compounds in all replicates in positive mode in ascending order of m/z

m/z	Retention time (min)
130.0964	0.250
177.0911	7.265
273.1811	3.324
301.212	4.341
324.2124	0.250
177.0911	7.265
301.212	4.341

Table 15 Common compounds in all replicates in negative mode in ascending order of m/z. Compounds in bold text were significantly different between treatments

m/z	Retention time (min)
71.014	0.73
71.014	2.69
73.030	1.10
87.009	**2.69**
111.009	**0.80**
112.986	**0.86**
114.056	0.66
115.004	2.69
131.083	**0.58**
133.014	0.73
146.046	**0.60**
147.066	1.56
156.991	0.57
161.046	**2.69**
179.014	**0.55**
181.072	0.59
191.020	0.62
195.051	0.61
217.003	**0.53**
219.051	2.85
245.043	0.63
271.081	0.57
277.033	0.62

287.038	1.07
289.072	**3.69**
293.124	3.70
297.020	2.56
333.059	0.62
337.077	0.74
353.053	**0.63**
374.992	0.52
377.070	**1.60**
383.155	2.44
443.176	2.44
467.107	0.66
501.026	0.52
532.980	**0.52**
577.098	**1.60**
625.160	0.56
659.015	0.52
674.992	**0.52**
690.969	**0.52**
132.030	0.60
147.030	0.82
192.981	**0.80**
209.030	0.63
307.012	**0.64**
517.003	**0.52**
533.454	**17.49**
581.124	0.57

The total ion count areas of the compounds in Table 15 were analysed for variance by one way ANOVA command where treatment was a fixed effect. There were 19 compounds out of 50 that were significantly different ($P < 0.05$) in area after the static WAM treatment. These P values are plotted in Figure 49, where $P < 0.05$ are under the horizontal line.

If this result were to occur by chance alone, only about three compounds rather than the 19 would be expected to exhibit a $P < 0.05$. A χ square test was doneto testif the probability of 19 chemicals out of 50 would make the treatments different from each other. The P value $< 2.2 \times 10^{-16}$ indicates that the three wine treatments were significantly different, but as is discussed shortly 'significant' is not the same as 'important'.

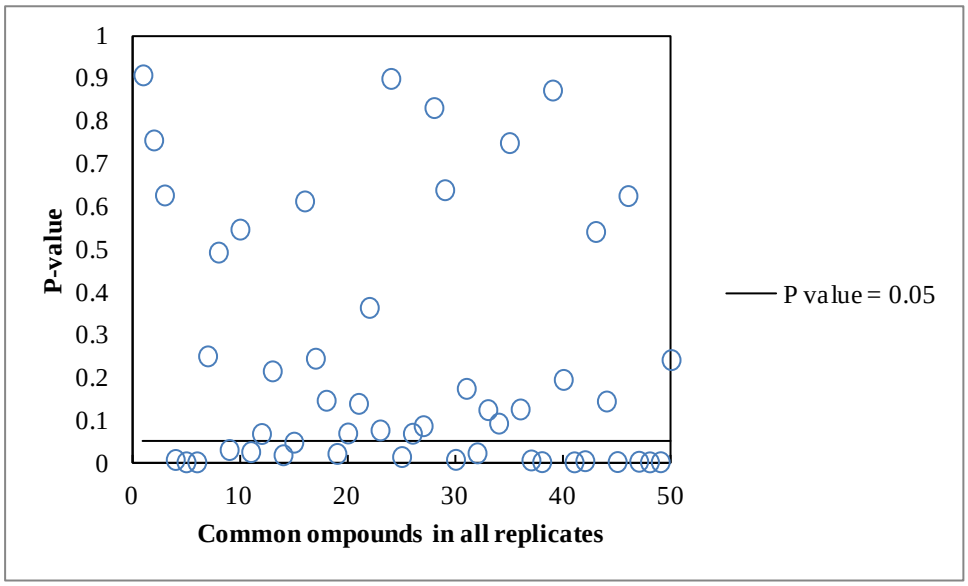


Figure 47 P values for area in compounds common to all replicates in negative mode

The chemicals that were significantly different between the three wine treatments are shown in Table 16. The potential identities of some chemicals were obtained from the METLIN Database by using $(M-H)^-$ mode (Siuzdak, 2013). In most cases, some chemicals exactly matched the m/z value in the database, but they are unlikely to be present in wine. (8-Hydroxy-3-chlorodibenzofuran maybe also be unlikely.) The true identities of these compounds would need to be confirmed by further analysis. The total ion area counts of the 19 compounds of interest are plotted in Figure 48, and although the differences were significant, can they be described as important differences?

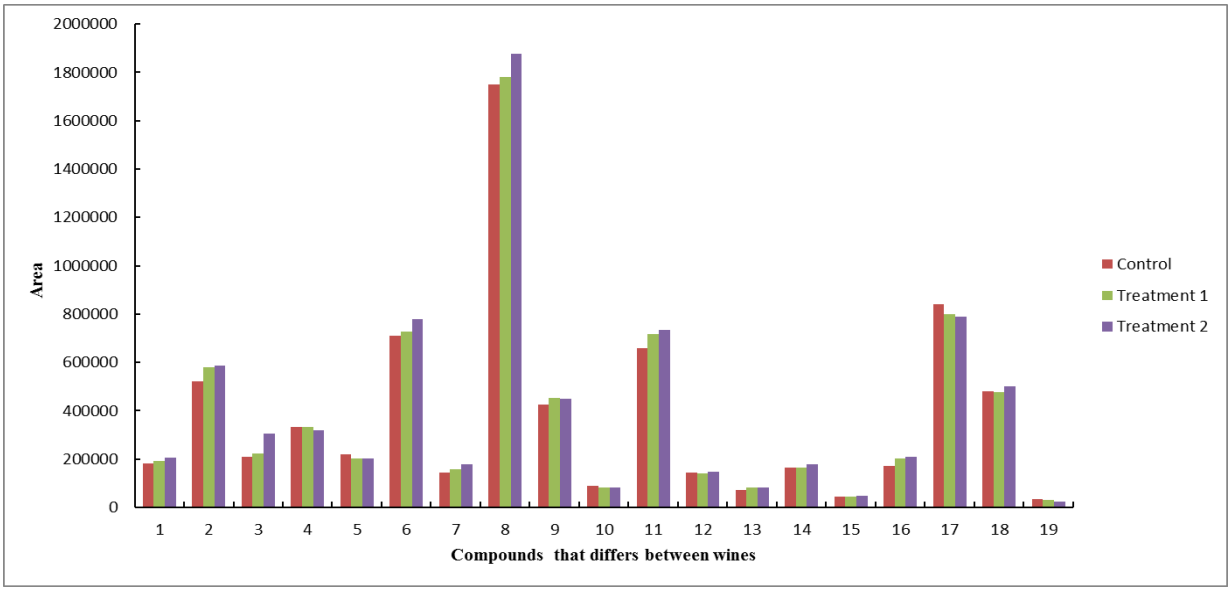


Figure 48 Compounds that differed significantly between the treatments

Table 16 Chemicals that were significantly different between the three wine treatments

Cpd.	m/z	Potential identity	Change in total ion count area from Control area (%)	
			Treatment 1	Treatment 2
1	87.0087	Pyruvic acid	6	13
2	111.0088	Furoic acid	11	12
3	112.9856		7	46
4	131.0826	Ornithine	0	-3
5	146.0457		-8	-9
6	161.0459		2	10
7	179.0141		10	22
8	217.0029	8-Hydroxy-3-chlorodibenzofuran	2	7
9	289.0718	Catechin	6	6
10	353.0531		-4	-8
11	377.0698	Anastain B (an anthocyanin)	9	12
	532.9802		-4	2
13	577.0983	Malvidin-3-(6'-malonylglucoside)	13	14
14	674.992		1	7
15	690.9693		-1	7
16	192.9813		18	21
17	307.0115		-5	-6
18	517.0034		-1	4
19	533.4539		-12	-32

Table 16 shows that for Treatments 1 and 2, most of the percent differences were usually in the same direction for each of the 19 compounds. That is to say, were consistently positive or negative with respect to the Control.

Thus, this selective analysis (800+ compounds narrowed to 50, then narrowed to 19) suggests that the WAM had a measurable effect. However, a less optimistic view is that the many compounds that were not present in all replicates (800+ less 50) represent chromatographic ‘noise’ that the above analysis has ignored to arrive at the ‘measureable effect’ conclusion. It is therefore important to repeat this analysis although the original UPLC-MS equipment was unavailable. Before doing that, another statistical analysis was performed on the short-listed 50 compounds to

show any differences. This was a principal component analysis.

The common chemicals (Table 15) in UPLC-MS negative mode analysed replicates were analysed by 6th PRIMER, principal component analysis (PCA) plot (Figure 49) was drawn on the base of area of the chemicals detected by UPLC-MS in negative mode (data not shown). The first principal component (PC1) and second principal component (PC2) explained 87.7% and 4.9% of original variability of this data set, respectively. In the plots of wine treated by different strengths of electric field, there is a marked separation between wine treated by the static WAM and the control wine (where the strength of electric field was 0). As to what compounds caused this shift is not clear, except to state that it is probably a combination of all the compounds listed in Table 15.

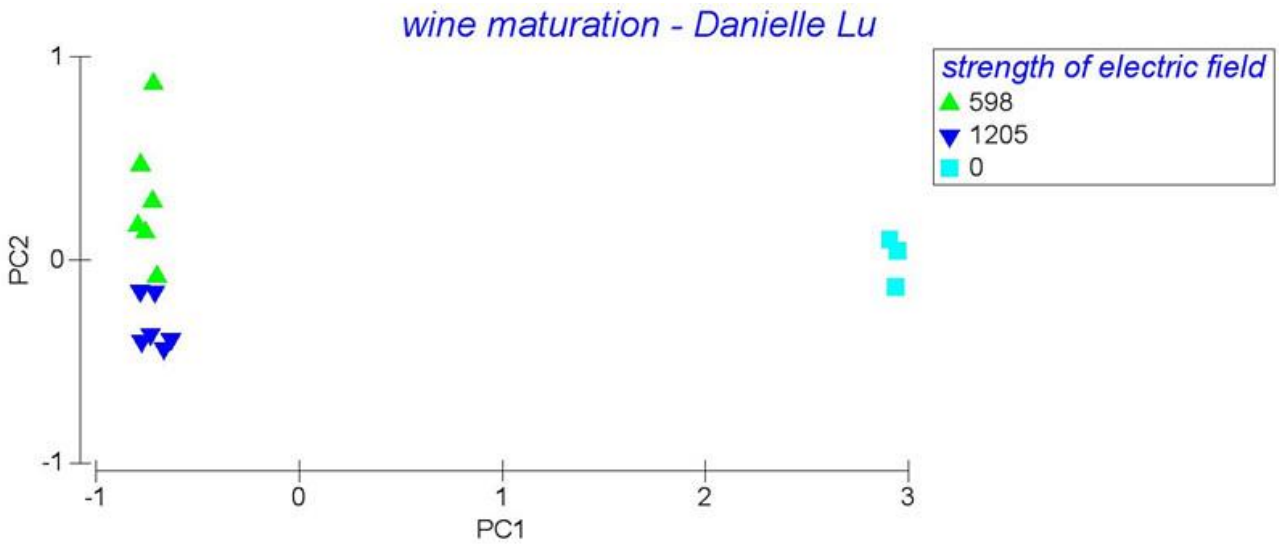


Figure 49 PCA plot of WAM treated wine and control

3.9 Chemical quantitation of the output from the static WAM

3.9.1 Introduction

In the previous section, there were indications that some compounds in wine were affected by exposure to a static WAM. These compounds were tentatively identified. In this section the work was repeated, with some minor differences, focusing on those compounds for which authentic standards could be obtained.

3.9.2 Methods

The chemicals used in this experiment are shown in Table 17.

Table 17 Chemicals used for LC-MS/MS

Chemical	Formula	Company
Citric acid monohydrate	$HOC(COOH)(CH_3COOH)_2H_2O$	Bio-lab (Australia) Ltd
Gallic acid	$(HO)_3C_6H_2CO_2H$	Sigma-Aldrich Co. LLC
DL-Malic acid	$HO_2CCH_2CH(OH)CO_2H$	BDH chemicals Ltd
3-Hydroxy-benzoic acid	$HOC_6H_4CO_2H$	BDH chemicals Ltd
Quercetin	$C_{15}H_{10}O_7$	Sigma-Aldrich Co. LLC
Tartaric acid	$HO_2CCH(OH)CH(OH)CO_2H$	BDH chemicals Ltd
o-Coumaric acid	$HOC_6H_4CH=CHCO_2H$	BDH chemicals Ltd
2-Furoic acid	$C_5H_4O_3$	Sigma-Aldrich Co. LLC
(+/-)- Catechin hydrate	$C_{15}H_{14}O_6 \cdot xH_2O$	Sigma-Aldrich Co. LLC
4-Chloro benzoic acid	ClC6H4CO2H	BDH chemicals Ltd
LiChrosolv ethanol	CH_2OH	Merck Ltd, NZ
Formic acid	CHOOH	Sigma-Aldrich Co. LLC

Three 750 mL bottles of Sauvignon Blanc wine were used, where Bottle 1 was destined for no exposure to an electric field, and Bottles 2 and 3 were destined for exposure at two levels. Table 18 summarises the treatments and the controls associated with those treatments. A 150 µL aliquot of wine was removed, and 150 µL of a 10 g L^{-1} chlorobenzoic acid solution in water was added to the bottle as internal standard. After thorough mixing by inversion, the spiked wine sampled into five 2 mL brown vials that were filled to the overflowing, and capped (A, C and E). The rest of the wine was treated by static WAM for three minute with the electric field off (B) or on (D and F), and used to fill five more vials for each bottle. This design used three different bottles, from the same

carton of 12, for the three treatments, Control, Test 1 and Test 2. At first sight this may appear to be a design fault, because the wine could vary from bottle to bottle, although these wines were sealed under Stelvin caps which are nominally identical unlike corks. However, it was reasoned that the act of pooling wine (Bottle 1 plus 2 plus 3) would add unwanted oxygen, such that experiments carried out later in time – up to one day later – would be with a potentially different wine because of oxygenation. If there were variations between bottles, at the very least comparisons can be (and were) made within bottle, that is to say A and B compared likewise C and D etc. In Table 18 row 2 (B), the Control wine was deemed to have been exposed to an electric field, hence the 'Yes' in Table 18 column 4: however, the exposure was a sham because voltage and frequency were both zero.

Table 18 Treatment of wine for LC-MS/MS

	Internal std. added?	Placed in WAM tubes?	Exposed to electric field?	Electric field details		Replication	Reference for text
				Field strength (V rms cm^{-1})	Frequency (Hz)		
Bottle 1	Yes	No	No	None		5	A
(Control)	Yes	Yes	'Yes'	0	0	5	B
Bottle 2	Yes	No	No	None		5	C
(Test 1)	Yes	Yes	Yes	598	3000	5	D
Bottle 3	Yes	No	No	None		5	E
(Test 2)	Yes	Yes	Yes	1026	3000	5	F

LC-MS/MS quantification was carried out on an Agilent 6420A (Figure 50) triple quadrupole mass spectrometer with a multimode ionisation source fed by an Agilent liquid chromatograph comprising a G1311C quaternary pump, a G1329B autosampler and a G1316A temperature controlled column compartment controlled by Agilent MassHunter software version B5. The analytical column was an Agilent SB-Zorbax C18 (2.1 mm × 50 mm × 1.8 µm) and was maintained at 30°C. The flow rate was 0.4 mL min^{-1} and the sample injection volume is 0.5 µmL.

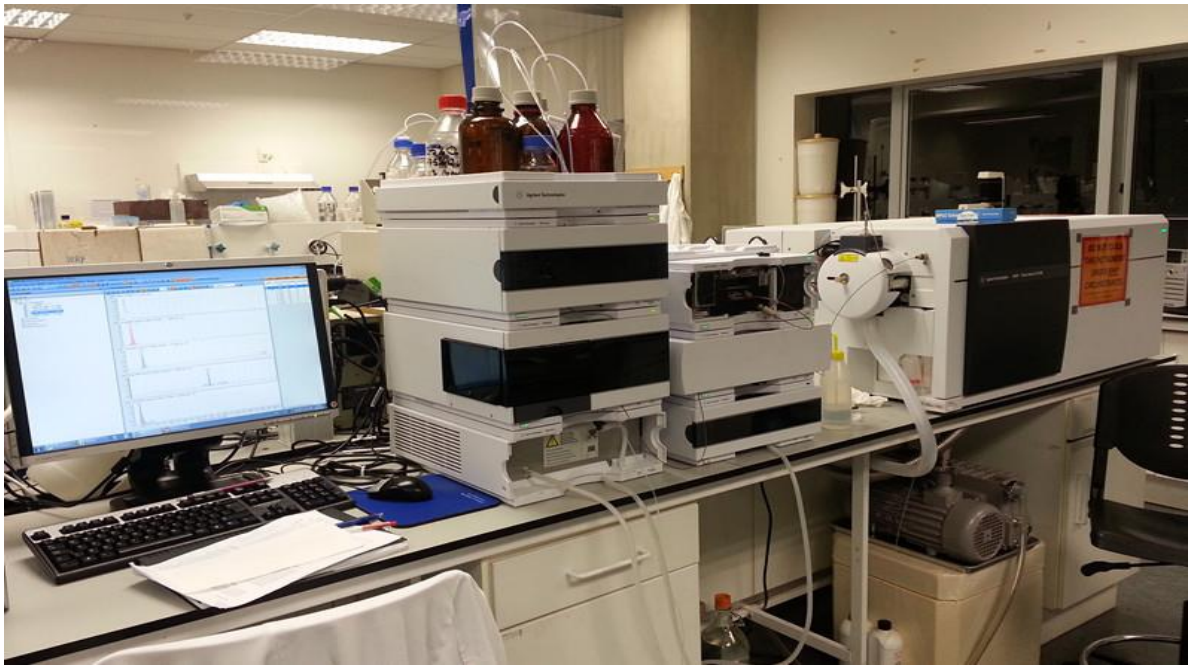


Figure 50 An Agilent 6420A LC-MS/MS

The gradient program for chemical quantification is shown in the Table 19. The multimode source was operated in mixed-mode negative ionisation with a gas temperature of 300ºC, APCI heater temperature of 200ºC, a drying gas flow of 5 L min^{-1} and nebuliser pressure of 414 kPa. The capillary voltage was 2,500 V, the charging voltage was 2,000 V and the needle corona current was set to 4 µA. The multiple reaction monitoring (MRM) setting were established using Agilent Optimise software in MassHunter version B5. These transitions are listed in Table 20. All transitions used a dwell time of 100 ms and a cell accelerator voltage of 7 V. The analysis was done in two days.

Table 19 Gradient program for LC-MS/MS phenolics quantification

	Mobile phase (%)	
Time (min)	A (2.5% methanol + 0.1% formic acid in water)	B (2.5% methanol)
0-2	100	0
10-12	20	80
14-22	100	0

3.9.3 Results and discussion

Except for malic acid, all the targeted chemical concentrations were in the calibration curve range (shown in Appendix I to VII A). The quantification results are shown in Table 21.

As explained earlier, the analysis was done over two days in such a way as to minimise oxidation effects. This required three bottles of wine that were not pooled. The overall detector responses for what were nominally replicates (A, C, E) increased in that sequence. Thus, an analysis of variance over the three bottles could not be done. However, the ratios B/A, D/C and F/E could give an insight into relative changes in concentrations of compounds (Figure 51).

Table 20 Transitions used for mixed reaction monitoring of organic acids

Compound	Molecular ion (m/z)	Product ion (m/z)	Fragmentor (V)	Collision energy (V)
Citric acid	191	111.1	86	5
Tartaric acid	169	125.1	102	9
Chlorobenzoic acid	155	111	82	9
Malic acid	133	115.1	80	4
2-Furoic acid	111	67.1	46	5
Hydroxybenzoic acid	137	93.1	68	9
Chlorobenzoic acid	155	111	82	9
Coumaric acid	163	119.2	84	9
Gallic acid	169	125.1	102	9
Catechin	289.1	245.1	124	9
Quercetin	301	151.1	138	17

Inspection of Table 16 suggests that furoic, hydroxybenzoic and coumaric acids may be affected by the electric field, although the pattern was variable with increasing electric field and therefore suspect. To formally test for statistical significance, unpaired t-tests assuming equal variance were done within-bottle for the compounds in Figure 51 (Table 21). Reassuringly, there were no significant differences for any compound in the A with B comparison. In Tests 1 and 2 only furoic acid was significantly different in the exposed treatments. Importantly, the effects were in different directions for the two tests: exposure increased concentration in Test 1 and decreased it in Test 2.

Table 21 Concentrations of chemicals in static WAM treated wine (mg L^{-1})

	Control			Test 1			Test 2		
	A	B	P value	C	D	P value	E	F	P value
Citric acid	62208	66017	0.08	77934	82256	0.19	109584	111534	0.43
Tartaric acid	397365	391682	0.61	448396	461089	0.39	738213	745989	0.86
2-Furoic acid	2732	2807	0. 43	2905	3129	**0.05**	3238	2845	**0.00**
p-Hydroxybenzoic acid	288	320	0.51	279	303	0.62	421	396	0.63
Catechin	2680	2604	0.44	2559	2570	0.91	3701	3563	0.16
Coumaric acid	18	9	0.06	13	12	0.95	21	14	0.70

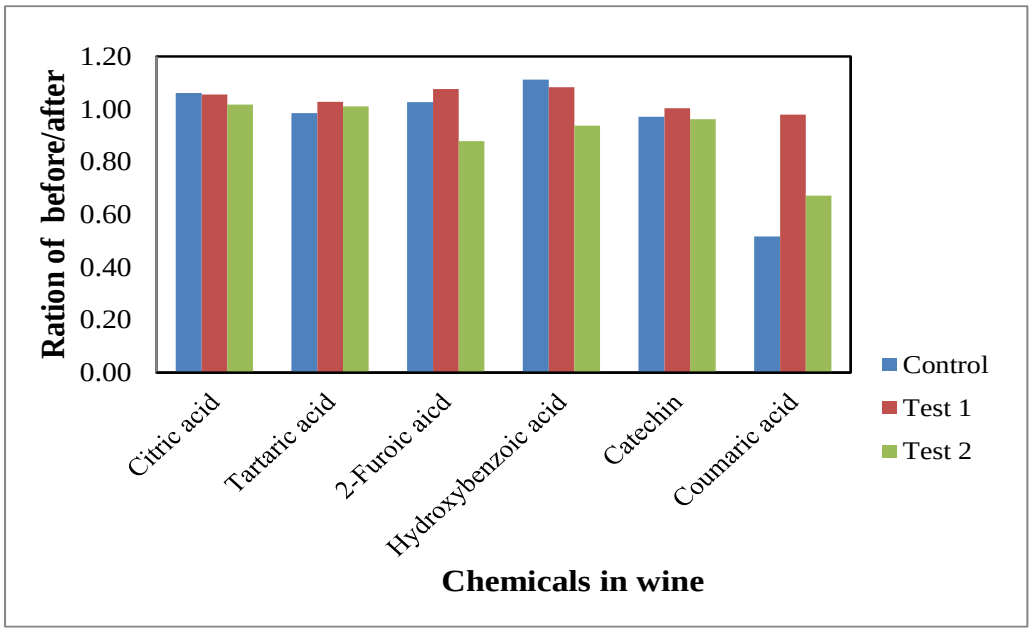


Figure 51 Comparison of chemicals in wine after treatment of WAM

In conclusion, the static WAM affected the concentration of only 2-furoic acid in Sauvignon Blanc wine, but in a non- consistent way: increasing the concentration in Test 1 and decreasing in Test 2. In the previous section, both Treatments 1and 2 increased the concentration. 2-Furoic acid is the degradation product of ascorbic acid (Chuang, Shen, & Wu, 2011; Goldberg, Hoffman, Yang, & Soleas, 1999). It is a volatile acid, the concentration of which increased during maturation in Madeira wine (Câmara et al., 2006).

Chapter 4

Conclusion

In these series experiments, white wines and red wines were treated by both flow-through and static wine ageing machine (WAM). Different frequencies and strengths of electric fields were used, and with different wine flow rates, zero in the case of the static WAM.

The wines were analysed by spectrophotometry in the wavelength range of 200 to 800 nm. The wine treated by both flow-through and static WAM did not show any change in colour.

Sensory analysis by discrimination was done with untrained panellists to compare WAM-treated and untreated wine. None of the sensory results were significant.

Finally, the compounds in wine were analysed by liquid chromatography to see if there were any significant differences. In the first of the liquid chromatographic studies there were about 800 compounds detected, not all of which were present in all replicates. There were however 50 that did fit that criterion and of those 50, 19 were significantly different, and those that could be tentatively identified were targeted in the second study. The result was not reproducible. Given this result it is now reasoned that the changes observed were random events. Indeed, normally distributed data can be expected to be 'significantly' different once in 20 events by chance alone. By this reasoning of the 800 compounds observed in the first chromatographic study, 40 (800/20) might be expected to be different. Forty is close to 50.

It remains to compare the present data with those of other researchers who claimed – perhaps validly – to have positive results. Table 22 summaries the experiments done recently for accelerating wine ageing in electric fields. Some of the treatments in wine/grapes have induced some physical and sensory changes in wine. In the different experiments, a wide range of different strengths of electric field, frequencies and duration were used. The starting point for my study was Talele et al. (2013) They exposed Merlot wine in the electric field of 282.8 and 565.6 Vrms cm^{-1} alternating at 3000 Hz in the flow-through WAM that I used for about 3 minutes. According to those authors, there was improvement of drinking quality of that unspecified in Merlot. Zeng et al. (2008) exposed young Cabernet Sauvignon wine in an alternating electric field of 424 Vrms cm^{-1} at

3000 Hz for 3 minutes. The treatment increased the free amino acids, alcohols and esters concentrations. The treated wine was described as softer and less astringent, implying changes to the phenolics.

Lopez et al. (2009) found the wine produced from a pulsed electric field-treated grape macerate resulted in clear demonstrations of higher concentrations of a wide range of phenols than in the control wine. For example, colour intensity increased due to increased anthocyanin concentrations, typically 35% higher. There is an important difference between a pulsed electric field and an alternating electric field. The alternating electric fields drew very little current, which could be ascribed to the resistance in the wiring and plates as the field changes rapidly. Thus, no significant energy enters the wine, and importantly neither the flow-through WAM nor the static WAM generated an increase in temperature in the present study. Using an alternating electric field, Zeng et al. similarly found there was no change in temperature in their work. In contrast, Lopez et al. (2009) reported that the energy input from their pulsed electric field was 2.1 kJ kg^{-1} of grape macerate and resulted in a small increase in temperature, about 2°C, at an unspecified temperature below 30°C. This important difference may explain the lack of effect seen in the present work, but does not explain the results obtained by Talele et al. and Zeng et al. The study by Cheng et al. (in Chinese) was short on detail, so their results add nothing to my understanding.

The negative nature of my results has been disappointing, and at several points I felt it would be best to cut my losses and seek another topic. However, my primary supervisor suggested that I continue and see the work through as thoroughly as possible. After all this work, I can conclude that with the wines used and the electrical configurations chosen, neither the flow-through nor static WAM worked to induce any clear changes.

Table 22 Summary of experiments with alternating electric fields applied to wine

Author	Wine/grape	Field strength (V rms cm^{-1})	Frequency (Hz)	Stationary/ Flow	Duration (min)	Major chemical/physical changes	Sensory changes
(Zeng et al., 2008)	Young Cabernet Sauvignon	424	3000	Flow	3	Free amino acids, alcohols, higher alcohols, esters concentration increased, total acidity decreased	Smell and taste tended to soft, astringency decreased
(Lopez et al., 2009)	Cabernet Sauvignon grape macerate	3535	122	Flow	< 1	Polyphenol index, anthocyanins and tannins concentration increased	Richer colour intensity
(Puertolas, saldana, Condon, Alvarez, & Raso, 2009)	Cabernet Sauvignon grape macerate	3535	122	Flow	< 1	Anthocyanins, catechin, flavonols concentration increased, total phenolic index increased	Richer colour intensity
(Zeng et al., 2004)	Rice	282	50	Stationary	180	Not determined	Not determined
(Chen et al., 2004)	Chixiazhu grape	3535/2121	?	Flow	3	Total free amino acid concentration increased	Less astringency, fuller body, smoother
(Talele et al., 2013)	Merlot	282.8/565.6	3000	Flow	3	Not determined	Improve drinkability
Present study	Merlot Cabernet	598	3046	Stationary	10	Not determined and no colour changes	None
	Sauvignon Blanc	598	3046	Stationary	10	Not determined and no colour changes	None
	Sauvignon Blanc	299	3046	Stationary	3	Not determined and no colour changes	Not determined
		598					
		906					
		1205					
	Sauvignon Blanc	299	3553	Stationary	3	Not determined and no colour changes	Not determined
		598					
		906					
		1205					
	Sauvignon Blanc	299	4145	Stationary	3	Not determined and no colour changes	Not determined
		598					

		906					
		1205					
	Sauvignon Blanc	598	3046	stationary	3	Furoic acid concentration increased?	Not determined
		1205				Furoic acid concentration decreased?	
	Sauvignon Blanc	424	3000	Flow	3	Absorbance change in 240-340 nm?	None
	Cabernet Merlot/ Pinot Noir	424	3000	Flow	3	None	None

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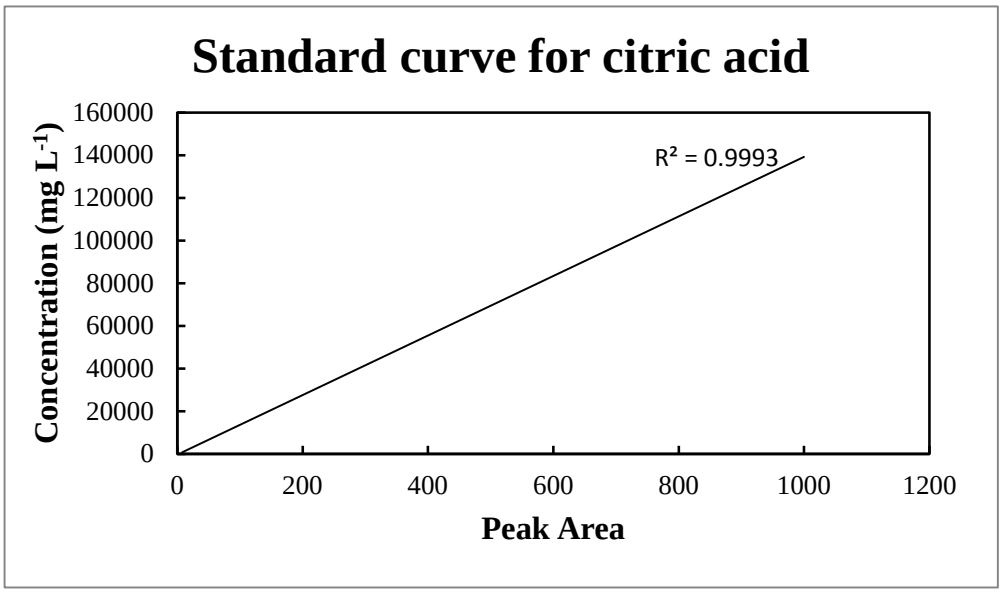
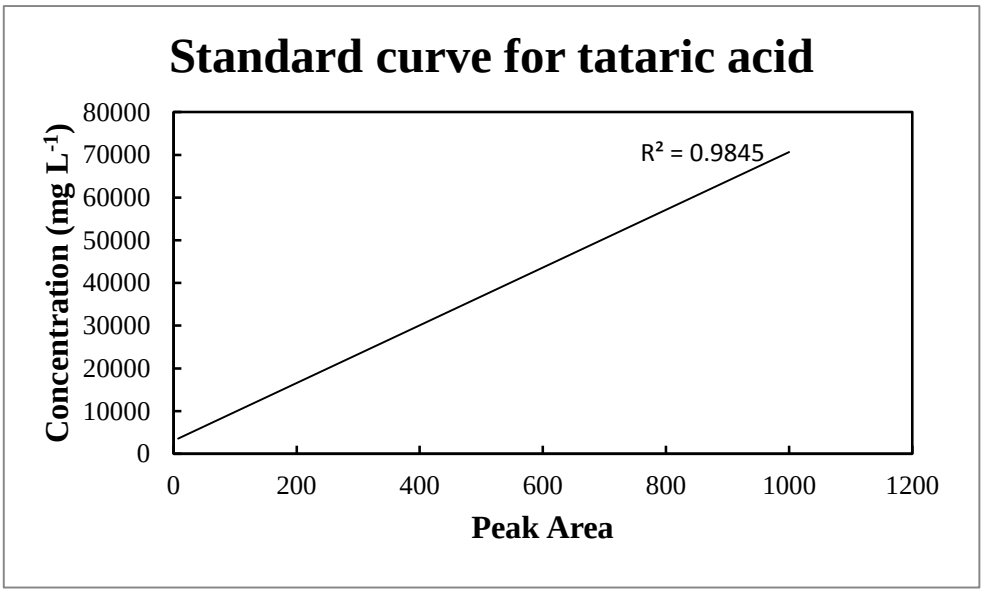
Appendices

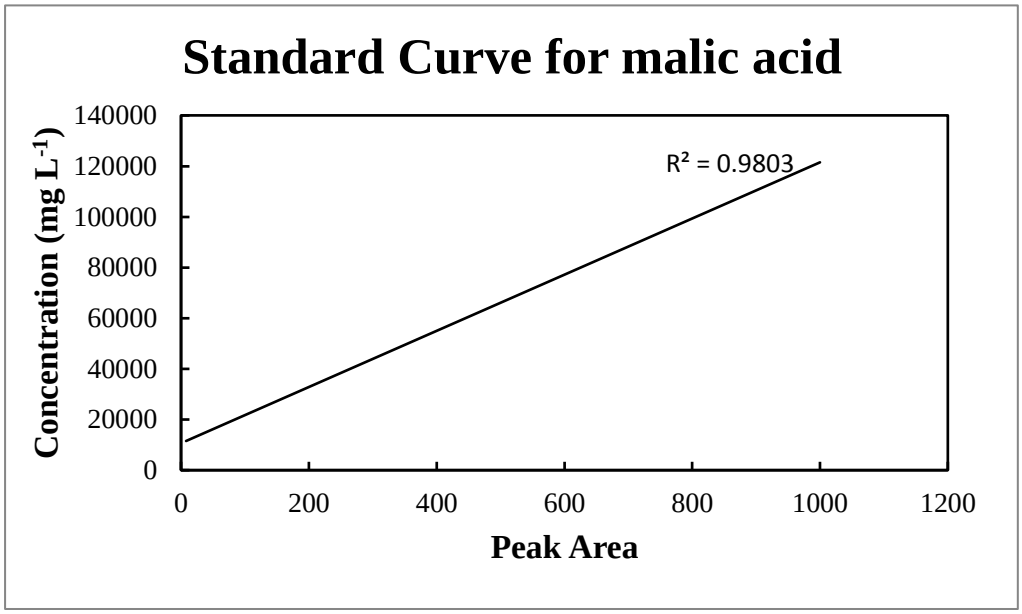
Calibration curve for acids and polyphenol quantification in wine



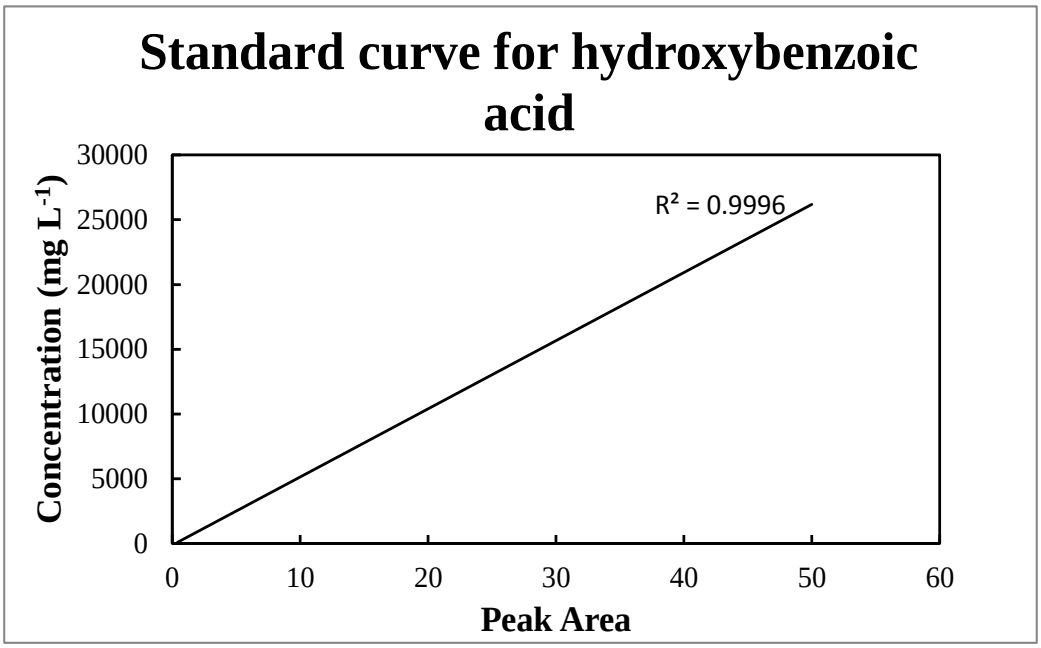
Appendix I A standard curve for citric acid



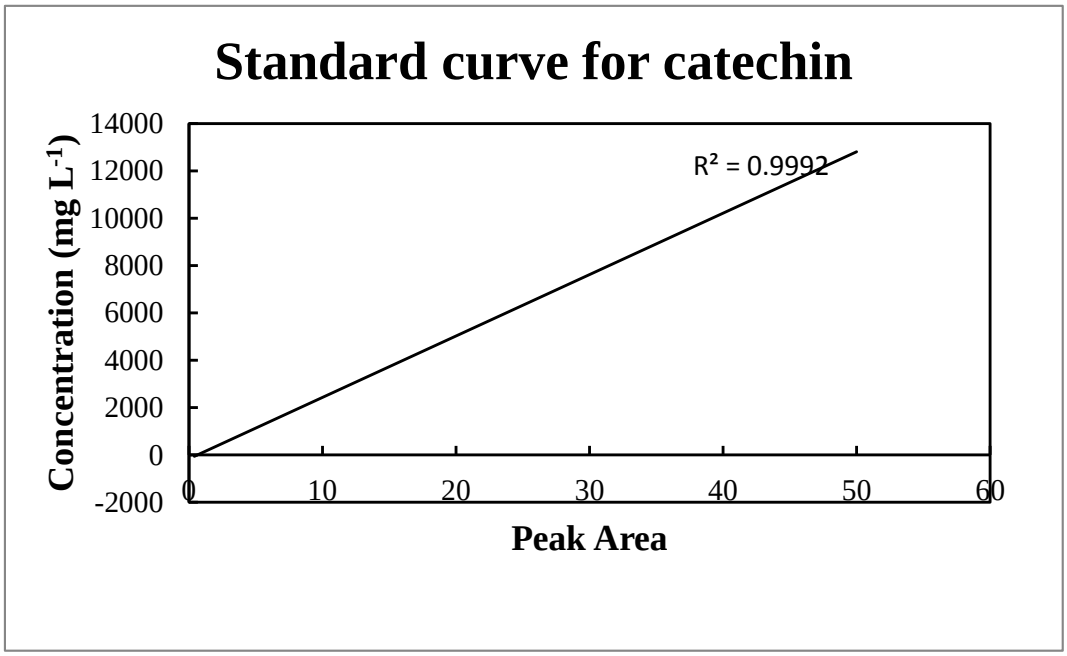
Appendix II A standard curve for tartaric acid



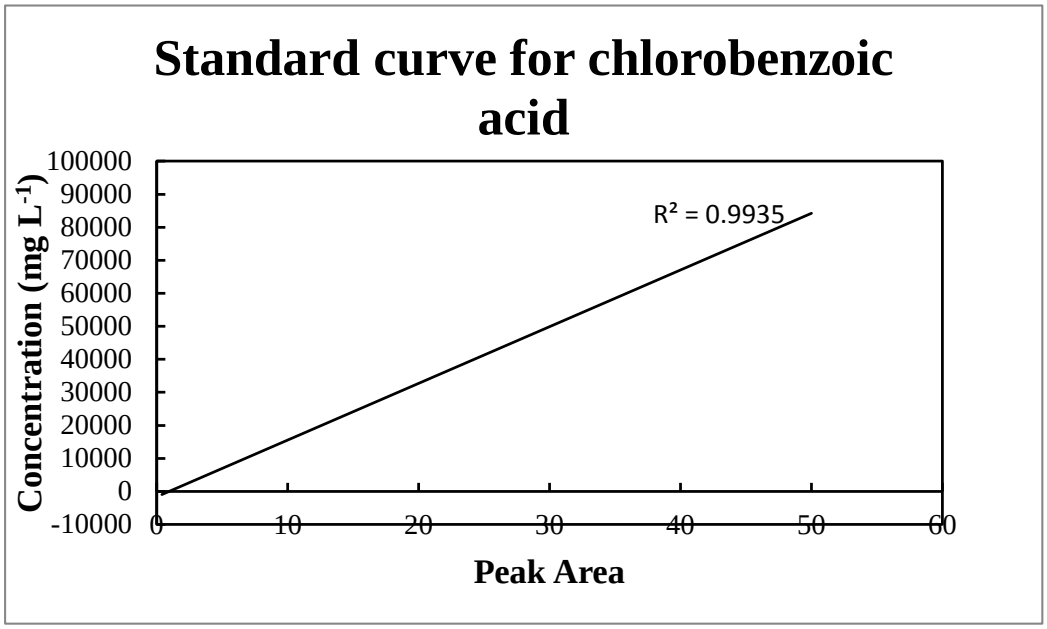
Appendix III A standard curve for malic acid



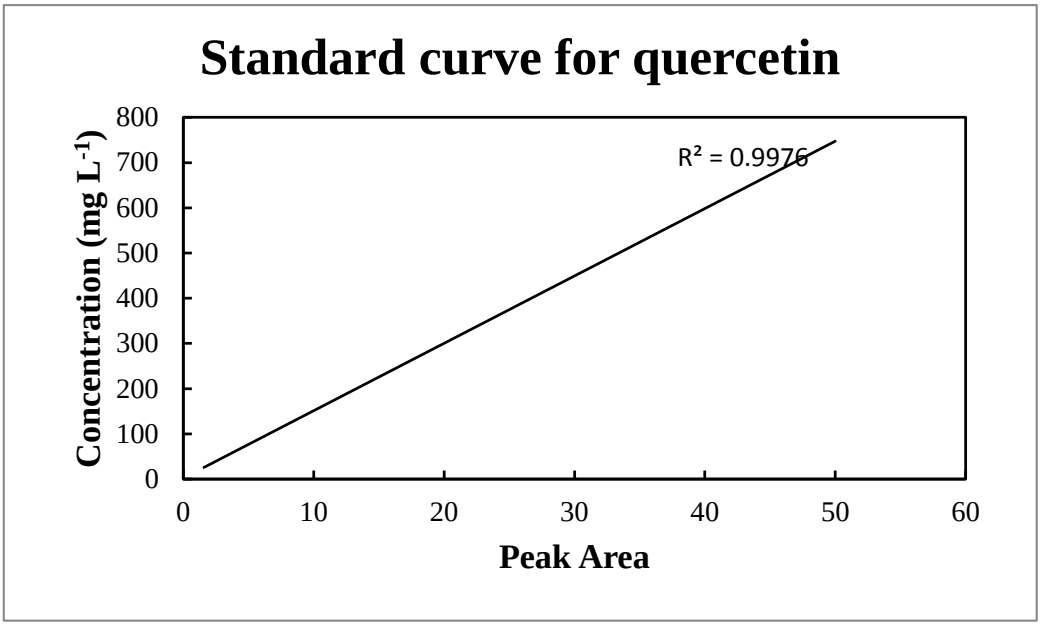
Appendix IV A standard curve for hydroxybenzoic acid



Appendix V A standard curve for catechin

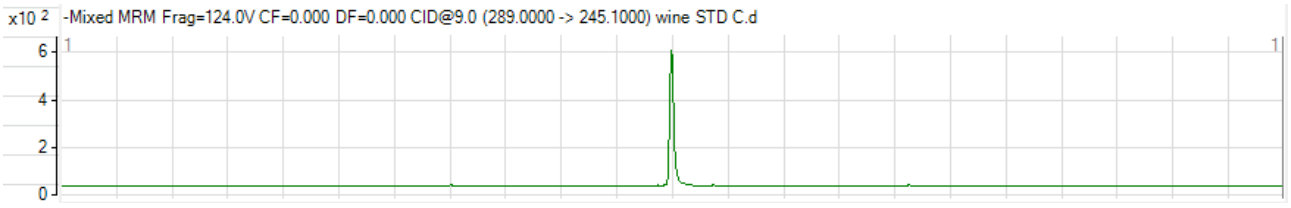


Appendix VI A standard curve for chlorobezoic acid

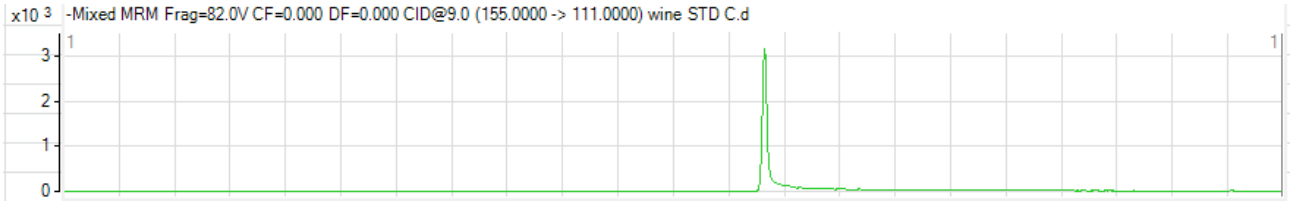


Appendix VII A standard curve for quercetin

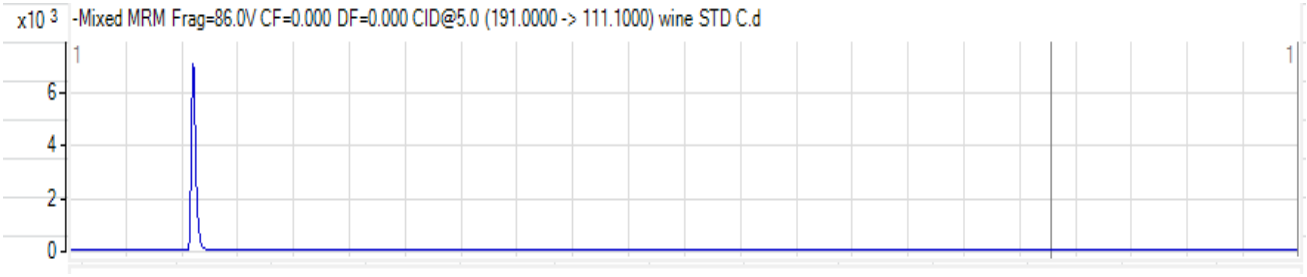
LC-MS/MS chromatograph of standards



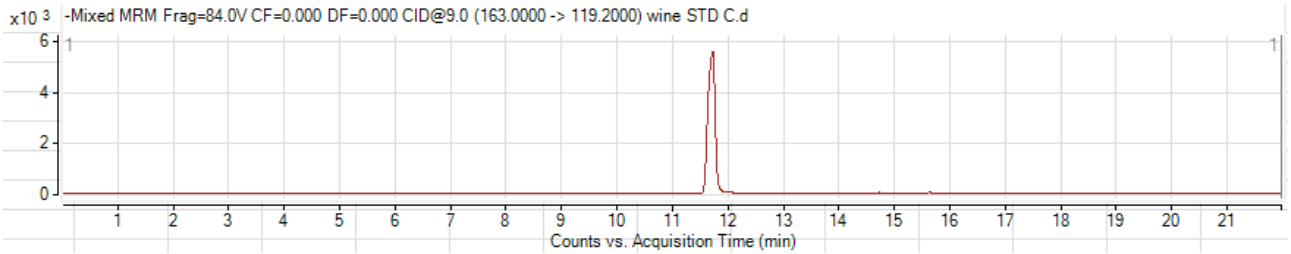
Appendix VIII A LC-MS/MS chromatograph of catechin



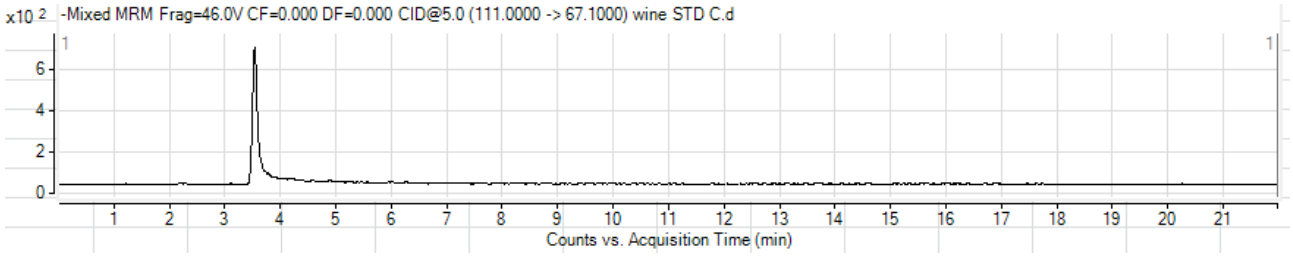
Appendix IX A LC-MS/MS chromatograph of chlorobenzoic acid



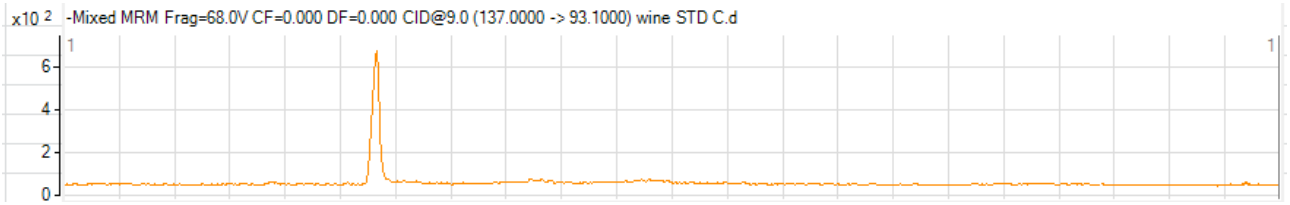
Appendix X A LC-MS/MS chromatograph of citric acid



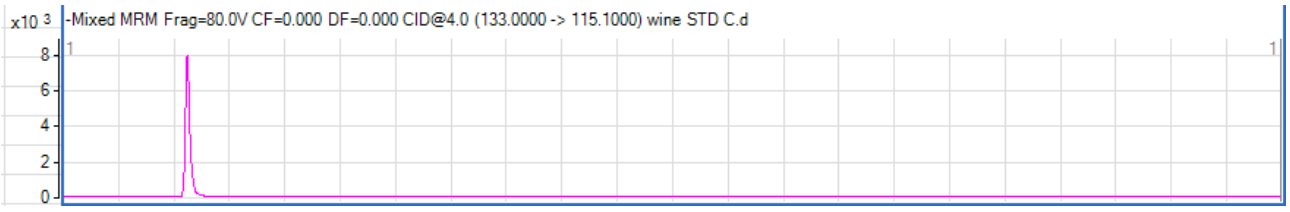
Appendix XI A LC-MS/MS chromatograph of coumaric acid



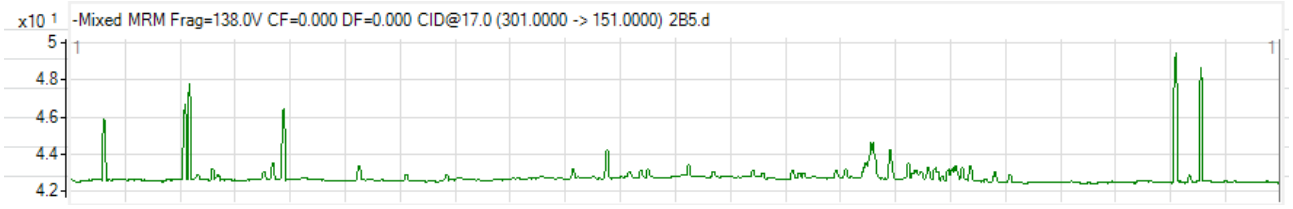
Appendix XII A LC-MS/MS chromatograph of furoic acid



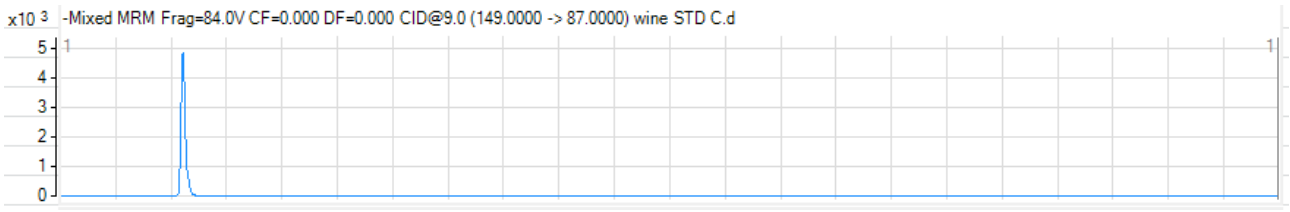
Appendix XIII A LC-MS/MS chromatograph of hydroxybenzoic acid



Appendix XIV A LC-MS/MS chromatograph of malic acid

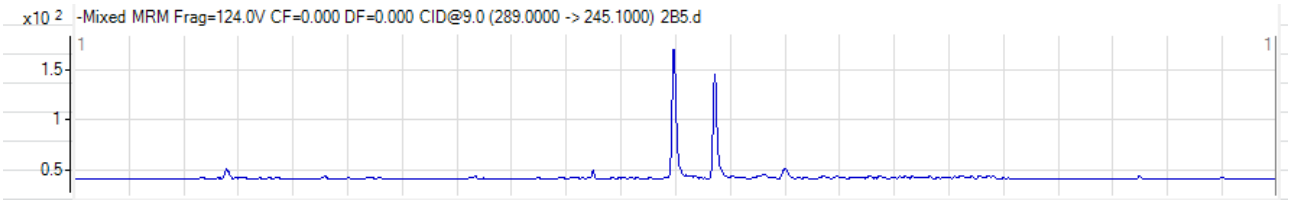


Appendix XV A LC-MS/MS chromatograph of quercetin

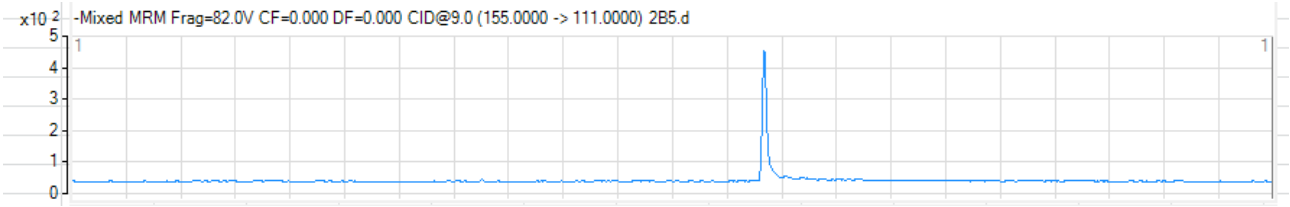


Appendix XVI A LC-MS/MS chromatograph of tartaric acid

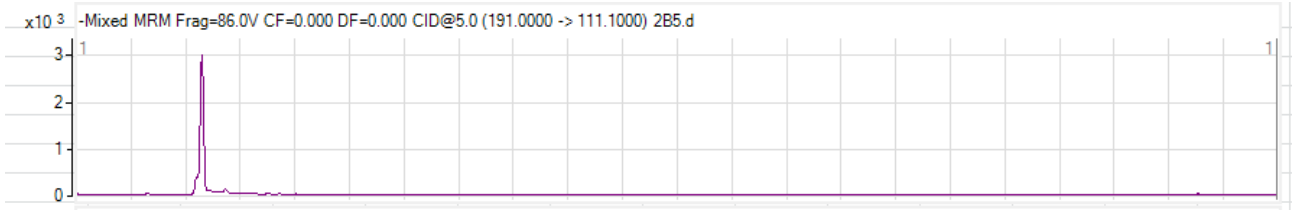
LC-MS/MS chromatography of compounds in wine



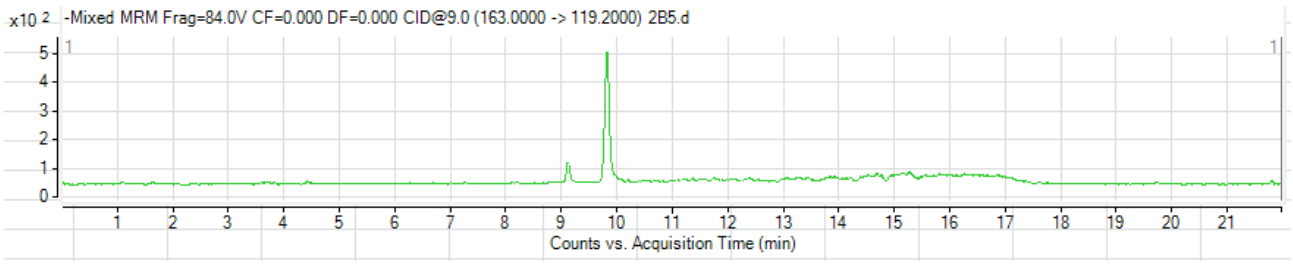
Appendix XVII A LC-MS/MS chromatograph of catechin in wine



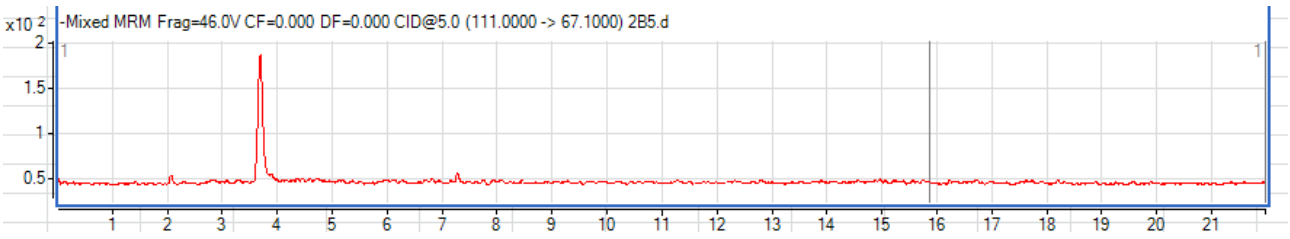
Appendix XVIII A LC-MS/MS chromatograph of chlorobenzoic acid in wine



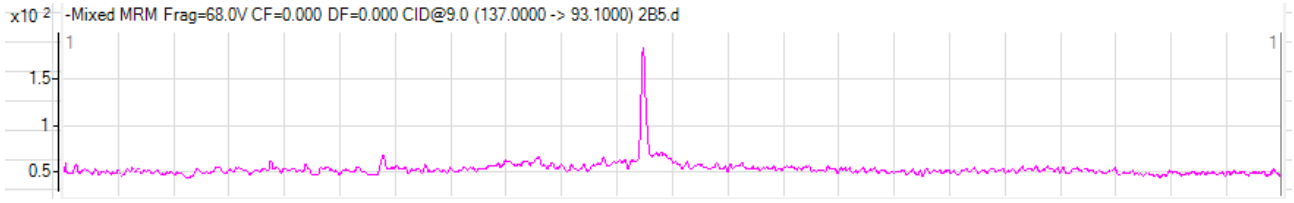
Appendix XIX A LC-MS/MS chromatograph of citric acid in wine



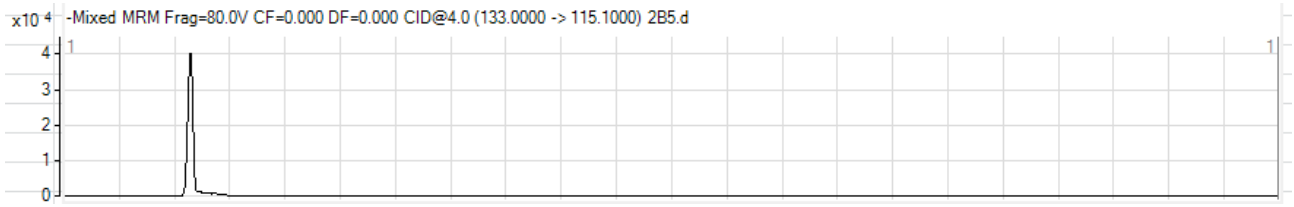
Appendix XX A LC-MS/MS chromatograph of coumaric acid in wine



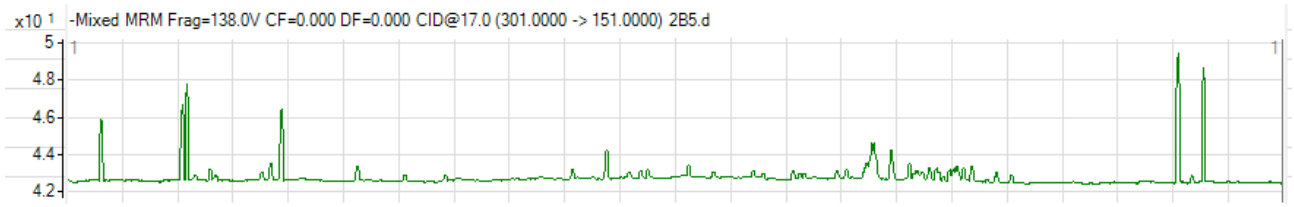
Appendix XXI A LC-MS/MS chromatograph of furoic acid in wine



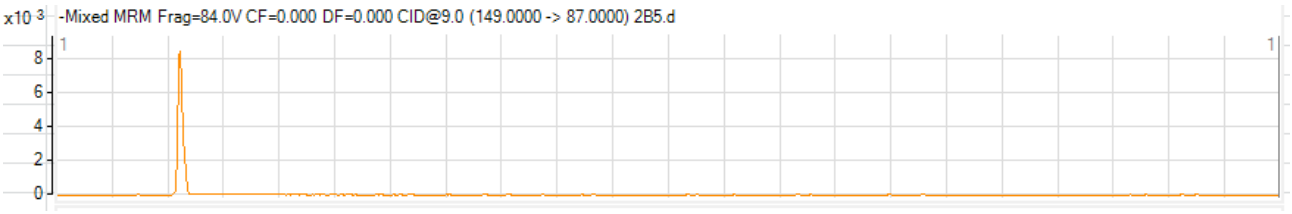
Appendix XXII A LC-MS/MS chromatograph of hydroxybenzoic acid in wine



Appendix XXIII A LC- MS/MS chromatograph of malic acid in wine



Appendix XXIV A LC- MS/MS chromatograph of quercetin in wine



Appendix XXV A LC-MS/MS chromatograph of tartaric acid in wine