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Light Wine

Technological and Legal Aspects of Alcohol Reduced Wine

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Abstract

The work investigates the technological and legal aspects of producing and commercializing alcohol reduced wine. For various reasons – related to health concerns, consumer fashions, and tax regimes among others – the global wine consumer market currently demands lower alcohol products. In response, industry and researchers have been working together to examine how to produce alcohol-reduced wines that maintain the technological features and organoleptic character of quality wine. As part of this effort, this work reviews the current state of the art in wine alcohol reduction technology, especially the stabilization of the wines during storage and their organoleptic quality. Through a series of cellar-based trials, the work shows that 50 mg/L of free SO_2 are efficient to avoid microbial spoilage in wines containing 4% and 8% (v/v), respectively. Moreover, based on a series of sensorial taste panels, the work makes recommendations on how to improve the organoleptic quality of alcohol-reduced wines, especially with regard to acidity, bitterness and body. At a different level, the work examines the legal framework for alcohol-reduced wines. It argues that once the actually available technology allows the production of quality alcohol-reduced wines and consumers desire such products, current OIV and EU regulations defining wine as grape fermented beverage containing at least 8.5% (v/v) may need to be revised. It is recommended to create a new legal category for 'light wines' containing between 4% and 8,5% (v/v).

Keywords: alcohol-reduced wine, osmotic distillation, ethanol perception threshold, 'light wine'

Resumo

O trabalho aqui presente investiga os aspectos tecnológicos e legais associados à produção de vinho de álcool reduzido. O vinho é uma das bebidas mais populares no mundo. Actualmente, por razões várias que vão desde a saúde, a moda, e o ambiente, a razões de ordem legal e económica (impostos sobre álcool), existe uma procura por um crescente número de consumidores de vinhos de nível alcoólico reduzido ou mesmo desalcoolizados.

O desafio por parte de quem produz, é obter produtos de qualidade, pelo menos, equivalente ao vinho produzido normalmente. Indústria, investigadores e enólogos têm vindo nos últimos anos a desenvolver e testar técnicas de redução do grau alcoólico através da manutenção da vinha durante todo a época e já no pós-vindima desde o período pré-fermentação até ao período pós-fermentativo.

Neste estudo propusemo-nos rever o estado da arte das tecnologias disponíveis para redução do conteúdo alcoólico, com particular destaque para a estabilização dos vinhos armazenados e a sua qualidade organoléptica. Através de uma série de ensaios experimentais em adega e laboratório, comprovámos que 50 mg/L de SO_2 livre são suficientes para evitar contaminações microbiológicas em vinhos com 4% e 8% (v/v) de conteúdo alcoólico.

Baseado em painéis de prova sensorial, neste trabalho fazem-se recomendações sobre como melhorar a qualidade organoléptica de vinhos de álcool reduzido, com particular a acidez, o corpo e sensação de amargo. A opinião sensorial dos vinhos por parte de provadores treinados, permite a produtores e investigadores optimizar técnicas e estratégias de vinificação. Lamentavelmente, algumas respostas permanecem por responder nomeadamente porque os vinhos utilizados não tinham como origem o mesmo vinho base sendo impossível uma avaliação real dos efeitos produzidos pela desalcoolização. No entanto, foi da opinião geral do painel que os vinhos em prova não tinham corpo e eram extremamente amargos e ácidos.

Numa outra perspectiva este trabalho explora a base legal de produção de vinhos de álcool reduzido. Havendo tecnologia disponível e vontade de consume por parte de quem bebe vinho, resta às entidades competentes, OIV e UE, rever os regulamentos que definem vinho como uma bebida produzida a partir da fermentação alcoólica de uvas contendo pelo menos 8.5% (v/v). Recomenda-se a criação de uma nova categoria para “vinhos leves” que contenham entre 4% e 8.5% (v/v). Quanto às questões legais dado que o limiar de detecção entre vinhos de diferente conteúdo alcoólico foi superior a 2%, poder-se-ia, eventualmente, alterar o valor mínimo para um valor ainda inferior. Para os consumidores de vinho esta redução do ponto de vista sensorial não deverá trazer qualquer alteração à opinião sobre o vinho e tendo em conta o mercado actual poderá significar um aumento da procura dos produtos. O hiato legal sobre ‘vinhos’ com valores de álcool inferiores a 8.5% poderia assim ser resolvido sem detrimento da qualidade do ‘vinho’.

Num futuro próximo seria crucial fazer novos painéis de prova com vinhos seguidos em todos os momentos da sua vinificação e desalcoolização, bem como testar de acordo com os resultados obtidos nos painéis novos lotes jogando, igualmente, com características chave como o doce, o amargo, o ácido e a sensação de corpo.

Palavras Chave: álcool reduzido, vinho, destilação osmótica, limiar de detecção de diferença entre conteúdo alcoólico, “vinho leve”

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Chapter 1. Introduction

Wine is one of the worldwide most popular alcoholic drinks. Since the beginning of wine making in ancient times, and Egyptians have been referred to produce wine already, that wine benefits have been mentioned. In recent years, a clear trend of increasing alcohol content in wines has been observed. While in the years 1970's the common alcohol content of wines was around 11-12 % (v/v) nowadays one most commonly find wines with alcohol levels between 12-14% (v/v) but it is not rare to find higher levels of 14,5-16% (v/v) (Ganichot 2002, Conibear 2006, Jones 2007).

Even though no particular cause as been appointed, anecdotic evidences show that this general increment is consistent with global warming across the globe and vine regions in particular (de Orduña 2010) and also the evolution in viticulture and vinification techniques and technologies have had an effect in such levels. Recent studies have shown that in the last decades global warming is causing an early ripening of the grapes and an increase of the sugar content at harvest and consequently higher alcohol content (Schultz and Jones 2010, Ashenfelter and Storchmann 2014). In average phenology stages have been anticipated in most vine regions, with reports mentioning harvest to happen 2-3 weeks earlier than in the beginning of the 20th century (Ganichot 2002, Stock et al. 2005, Nemani et al. 2001). Nevertheless, these dates should be looked at carefully as their anticipation also results from changes in vinification styles and strategies (and not only grape maturity).

Drinking demands for highly concentrated and aromatic wines by consumers has also led to an increase in alcohol content, as it requires more matured grapes and thus with higher sugar concentrations. Both in the vineyard and in the cellar, winemakers push the grapes towards a higher production of aroma and taste, and consequently alcohol. When analysing the available commercial fermentation yeasts one is subjected to a wide range of possible different aromas and tastes to choose from, that go beyond in many (if not most) occasions from the "natural grape varietal characteristics". The contradiction is here the queen, on one hand consumers demand concentration on the other hand demand *terroir* - and they may not be compatible in the same bottle…

The higher the alcohol and the concentration of the wines the more difficult it is to drink and appreciate them on a regular basis. As any other beverage, a “strong” wine becomes boring and overwhelming, not desirable for drinking very often. New social and health trends among, mostly cosmopolitan, consumers, looking for biological and local products, free of chemicals and artificial aromas and of gluten and of sugar and finally of alcohol, combined and reinforced by state health and self-responsibility campaigns about the consequences and dangers of (excessive) alcohol consumption are leading to a progressive change in consumer demands and habits. Changes in countries legislations on legal alcoholemia for driving and alcohol taxes pay here, also, an important role “forcing” winemakers to look for lower alcohol level alternatives. Moreover, in countries where taxes are levied according to alcohol content, the higher the content the higher the prices despite the wine quality.

Research wise alcohol reduction is not a new topic (e.g. Bonneau 1982, Bui et al. 1986, Schmidtke 2012), and many studies have focused in the improvement of new techniques and technologies for reducing alcohol content of wines along the whole winemaking process, starting already in the vineyard but also pre-fermentation, during and after-fermentation. The main challenge has been to produce low alcohol wines conserving the organoleptic balance and quality, that can convince consumers.

Can the “perfect bottle” of wine be a reduced alcohol one? Or is this a chimaera? Are there technologies/techniques for alcohol reduction that allow to maintain wine organoleptic characteristics and quality? Are the wine consumers ready to give “reduced alcohol wine” a chance? Am I ready?

The main goal of this thesis is to evaluate the impact of alcohol reduction on the organoleptic quality and balance of the wines in terms of sensorial perception and acceptability as well as their long term preservation as a limitative factor for their commercial value. To accomplish this, several questions will be addressed:

1. Is the alcohol reduction in the wine perceived by the consumers? what is the “perception threshold”?
2. If so, how is the reduction sensorially perceived? and is it positive or negative?

3. Can the reduced alcohol wines be preserved the same way as the “normal” wines?

This thesis will be presented in different chapters. The first one will include a literature review directly connected to the objectives of the research. Wine legal framework in terms of wine content, sensorial impact of alcohol in wine, comparison of different alcohol reduction techniques and consumer perception and demands will be explored. A second chapter will follow with Material and Methods applied as well as their pertinence to the research. The third and fourth chapters will describe the main results obtained and a discussion of their relevance in view with the objectives, respectively. A final chapter will follow with a summary of the main conclusions and their importance to the future of the wine industry.

Chapter 2. Alcohol reduced wines

2.1 Wine composition and ethanol effect

Wine composition depends greatly on grape variety (Bejerano and Zapeter 2013) but also on viticultural management that interferes with vine metabolism and on the vinification processes, namely during fermentation as a function of the yeasts and bacteria present and their respective metabolisms (Keyzers and Boss 2010, Boss et al. 2014). Being a very complex product, chemical analyses have repeatedly proven the presence of hundreds of distinct molecules that can affect both positively and/or negatively the quality of the wine.

During alcoholic fermentation carried out by yeasts, glucose and fructose are transformed/metabolised into various products, mainly alcohols, and other secondary products like poliols, fat acids, organic acids and many volatile compounds (e.g., thiols). These molecules, many formed from precursors (e.g. volatile alcohols, esters, acids, terpenes, and carbonyl compounds) already present in the grape, can undergo transformation during wine ageing altering wine characteristics and complexity perception (Ebeler and Thorngate 2009, Robinson et al. 2014). All together these compounds form a very complex matrix of the aroma and flavour of the wine (Fig. 1).

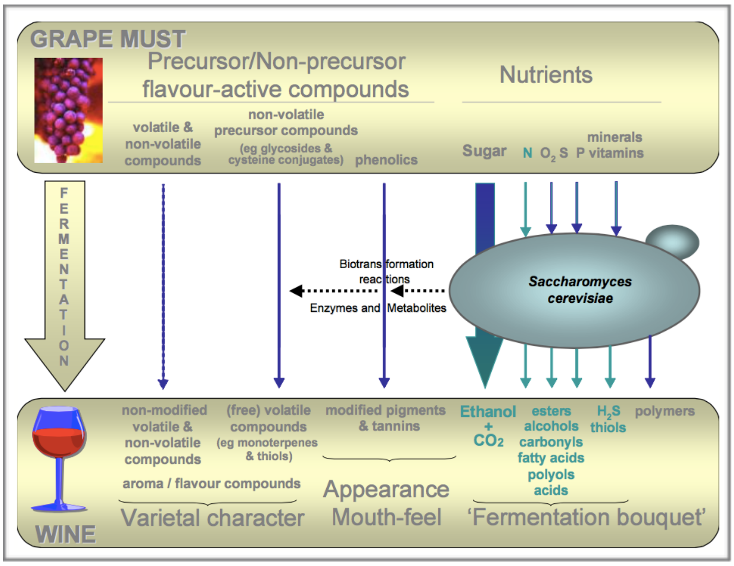


FIGURE 1. MAIN CHEMICAL COMPOUNDS OF GRAPE MUST AND WINE SUBJECT TO BIOTRANSFORMATION BY YEAST (ADAPTED FROM BELL AND HENSCHKE 2005).

2.1.1 Phenolic compounds

Phenolic compounds are the major non-volatile group of compounds, and have a great and studied influence in the perception of wine quality. Better known nowadays for their antioxidant properties with proven benefits to humans' health, phenols play an important role in the colour (specially in red wines), astringency and bitterness of the wines. This group is divided into two subgroups the flavonoids and the non-flavonooids. The first one includes flavonols, anthocyanins and tannins and the second the phenolic acids and stilbenes.

White wines are essentially composed by phenols originating from the flesh of the grapes (white wine vinification usually avoids the use of the grape skins) such as gallic acid, catechin, epicatechin, gallocatechin and catechin gallate, procyanidin, and hydroxycynammic acids. Red wines, in addition to the phenols mentioned for the white wines, due to varietal composition and vinification processes using the maceration of the skins, the list also includes flavonols like leucocyanidin, quercetin, anthocyanins and tannins (Peynaud 1984, Margalit 2004).

Anthocyanins, responsible for the colour in red wines, react with tannins forming polymeric compounds (Remy et al. 2000), that bind with proteins contributing to a reduction of astringency in wine during aging (Singleton 1992). Tannins are probably the most important compound contributing to astringency (drying, rough feeling), reacting with the salivary-mucoproteins and proline-rich proteins of the saliva. The first when bound to tannins precipitated reducing lubrication with an increased friction sensation in the mouth (Green 1993), whereas the second bind favourably with tannins (Haggerman et al. 1998)

2.1.2 Polysaccharides

Polysaccharides, carbohydrates chains of various lengths, constitute the main group of macromolecules present in the wine. Mannoproteins originate from cell walls of the microorganisms present in the grapes, must and wine responsible for the metabolisation of many other compounds especially during alcoholic fermentation and arabinogalactan proteins (AGPs) and rhamnogalacturonan II (RG-II) originate from the hydrolysis of the peptide chains of the grape cell walls.

As any other wine compounds polysaccharides also interact with different molecules altering wine properties. These molecules act preventing protein haze in white wines (Waters et al. 1994) and protectively in tartrate stability (Gerbaud et al. 1997). Proteins react with anthocyannins contributing to colour stability (Escot et al. 2001). The presence of mannoproteins has been related to body and fullness sensation of the wine (Vidal et al. 2004, Guadalupe et al. 2010) and

2.1.3 Sugars

Glucose and fructose are the two main sugars present in the grapes and wine, though glucose is metabolised to a great extent during alcoholic fermentation by yeast. Residual sugar is thus dominated by fructose, and not perceived as sweet in most common dry wines with less than 2.0 g/L.

Sweetness is wines is caused by other compounds influence such as ethanol, acids and also tannins interaction.

2.1.4 Nitrogen compounds

Within the nitrogen compounds group, it is included the amino acids, peptides and proteins but also ammonium compounds. Nitrogen compounds in the must play a crucial role to yeast growth and metabolism but are also known to cause sluggish or stuck fermentations if in insufficient level (Bisson 1999).

Most abundant amino acids present in wine are alanine, aspartic acid, glutamic acid, glycine, serine and threonine. Amino acids constitute a major source of yeast available nitrogen (YAN) for yeast, but not all amino acids are equally valuable (ammonium, glutamine and asparagine are preferred sources of nitrogen whereas proline and urea are not, being considered poor nitrogen sources). These are important metabolic precursors of higher alcohols, a major group contributing to wine aroma.

Proteins in wine come both from the grapes and from autolysed yeast. Proteins, present in low concentrations, play however important roles in wine clarity and stability (reviewed in Ferreira et al. 2002a). Protein instability, especially, in white wines is considered a fault. The denatured protein can precipitate forming a deposit and unattractive haze that reduces wine commercial value. This instability is induced by low pH and high ethanol and polysaccharides content (Lagace and Bisson 1990, Mesquita et al. 2002). But protein presence can also be positive as it is involved in foam formation and stability in sparkling wines.

There are not many studies about the influence of proteins to the aroma and flavour of wine.

2.1.5 Organic acids

Organic acids, such as tartaric, malic, citric, gluconic, lactic, succinic and acetic, derive from grapes but also from microbial metabolism (Ribéreau-Gayon et al. 2006). The most abundant are tartaric, malic and citric acids, respectively. Phenolic acids are also present but in much lower concentrations, including the cynamic and benzoic acids.

Tartaric acid in particular interacts with other wine compounds, like proteins, that have been described to be part of the tartrate crystals composition. Succinic acid is the main carboxylic acid that originates from alcoholic fermentation and interferes with wine aroma (Ribéreau-Gayon et al. 2006).

Sourness is associated with the concentrations of various acids, but is also affected by other factors like pH, sugar and ethanol concentrations. Different acids affect differently the perception of sourness; for the same acidity perception of sourness increases with malic, tartaric, citric and lactic acids, respectively (Fischer and Noble 1994). Taste and mouthfeel sensations are also affected by acids. Sweetness is decrease with increasing acidity levels, and tartaric acid was described to suppress fructose sweetness (Zamora et al. 2006).

2.1.6 Wine composition: aroma and flavour

Aroma, flavour, mouth-feel and wine appearance are in general terms influenced by four major sources of compounds: 1) primary grape derived compounds resulting from plant cell metabolism; 2) secondary grape derived compounds modified by processing; 3) fermentation derived compounds; and 4) ageing derived compounds formed during wine maturation (Rapp and Versini 1991).

The aroma of varietal wines results of the combination of odour-active aroma compounds that originate from distinct sources according Ferreira et al. (2002b): 1) distinctive variety-specific volatile compounds; 2) non-odorous non-volatile precursors of glycoside or cysteine conjugates are transformed by acid-catalysed or enzymatic hydrolysis and 3) amino acid metabolised by yeast. The sugar metabolisation of yeast during alcoholic fermentation mainly contributes to the formation of higher alcohols, fatty acids, esters, carbonyls, S-compounds and several organic acids while nitrogen metabolism is originating higher alcohols and esters and additionally hydrogen sulphide, thiols/ mercaptans and monoterpenes, also belonging to the volatile compounds group.

There are four basic distinct classes of varietal aromas comprising monoterpenes, C13 norisoprenoids, methoxypyrazines and sulphur compounds (with a thiol function) (Fischer 2007), most of them present in the grape in their non-volatile bound form and hence no odour. *Monoterpenes* are found in higher concentrations in white wine varieties, like Riesling, Gewürztraminer, Arinto and Loureiro, eliciting floral aroma. They exist in the grapes as glycol-conjugates being liberated during fermentation by acidic hydrolysis and glycosidic enzymes, and recently it was suggested that yeast and bacteria can also produce monoterpenes (Zoecklein et al. 1997, Carrau et al. 2005). Many studies have focused in the odour thresholds that vary according to the wine matrix, but also from person to person. Common monoterpenes are linalool giving floral with citrus notes; geraniol identified by geranium notes and wine lactone recognised by a sweet coconut like spice aroma.

C13 norisoprenoids originate from oxidative cleavage of carotenoids, and are mostly present as glycosides in the form of monoglucosides. ß-damascenone, present in Chardonnay and Riesling wines, is a very powerful aroma compound with really low odour threshold (~50 ng/L in a model wine, Escudero et al. 2004), that depending on the concentration varies aroma from lemon balm at low concentrations to apple, rose and honey notes at higher concentrations (Fischer 1995). With higher odour thresholds and characteristic of Touriga Nacional red variety is ß-ionone, brings a violet aroma to the wines. One other example is 1,1,6-trimethyl-1,2-dihydronaphtlanene (TDN), linked to the ageing flavour of Riesling described as petroleum, kerosene notes.

Methoxypyrazynes, despite the low odour thresholds are very potent odourants, easily identified in wines made of Cabernet Sauvignon and Sauvignon blanc. Green bell pepper is the descriptor that better qualifies for these compounds, and is by many considered a marker for unripe grapes in the Bordeaux wines, and highly appreciated in New Zealand Sauvignon blanc wines. But also vegetative, herbaceous and capsicum-like aromas are commonly identified in association with methoxypyrazynes. When present in higher concentrations can elicit unpleasant odours in wine, considered defects.

The last group, many times pointed as responsible for a wide range of off-flavours like rotten eggs caused highly volatile thiols such as H_2S or like onions and burnt rubber caused by methyl and ethyl sulphides, is the *sulphur compound with a thiol function group*. Among the highly volatile thiols there is also dimethyl sulphide (DMS) that in very low concentrations can bring positive asparagus, cooked corn aromas (Rauhut 1993). In Sauvignon Blanc were identified some volatile thiols contributing to its varietal aroma: 4-mercapto-4-mthylpentan-2-one (4-MMP) smelling like black currant, boxwood and broom; 3-mercapto-hexan-1-ol (3-MH) and its acetate ester (3-MHA) linked to the tropical aromas often exhibited by the wines. In the grapes fruits these thiols are only present as odourless cysteine conjugates that are released by hydrolysis revealing the hidden tropicality of the grapes.

Other compounds contribute to wine aroma. Higher alcohols to my knowledge no studies reported clear influence of these compounds to wine aroma; esters impart fruity and floral aromas, volatile fatty acids like acetic acid in small concentrations elicits wine complexity, however above certain levels become unpleasant; aldehydes in particular acetaldehyde (ethanal) has a characteristic green apple aroma, and diacetyl a buttery aroma, not always positive.

The revelation and development of these aromas are highly dependent on the raw material but also on vineyard and ultimately oenology practices (for a comprehensive list of the most important aroma compounds in the wine and the impact of grapes and microbial activity see Fischer (2007) Table 11.2).

2.1.7 Sensorial and quality assessment in wine

Wine quality can be described by a group of sensory attributes of aroma, flavour, mouthfeel, and finish (Lecocq and Visser 2006, Benfratello et al. 2009, Jackson 2009). A general definition of quality in foods and beverages, wine included, by the International Organisation for Standardisation (2000) is ‘the ability of a set of inherent characteristics of a product, system or process to fulfil requirements of customers and other interested parties’. Combining both definitions leads us to a better understanding on how wine quality can be perceived and later described, and ultimately transposed to enhancement of the wine quality. Charters and Pettigrew (2007), interestingly point two quality dimensions based on consumer experiences: the external one, based on the grapes, production and marketing; and the internal one, based on attributes like pleasure, appearance, aroma and taste.

Tasting panels have long been used for sensorial and quality assessments. But each person is different both genetically, culturally and psychologically, increasing the variability in opinion about the same tasted product, that is having a different perception for the same tested attribute. The heterogeneity of the panel can thus bias the results and the given interpretation (Lawless et al. 1997).

Many studies have analysed the best way to conduct a tasting panel coming up with several basic principles that should be taken in consideration for a good sensory evaluation practice, including '(1) paced testing, (2) blind and randomised presentation, **(3)** use of blind replicate samples to test judge reliability, (4) statistical treatment of data including elimination of outliers, and (5) independence of judgment from inter-judge influence' (Goldwyn and Lawless 1991).

In the wine market, in particular, consumers are highly influenced by the word-of-mouth of the wine experts and critics, like Jancis Robinson or Robert Parker, just to name two among the long list of the names known worldwide. Saying so wine quality is part of the wine expert job, but in many situations, such as academic and market studies the need to have a large group of panellists is common. In preliminary phases in which new products are being tested and specific positive and negative characteristics are to be identified the use of a trained panel is advised. Gawel and Godden (2008) tested the ability of experience tasters to consistently rate wines quality and observed a great variability between individuals and also for white wines (when compared to red wines).

Wine perception is directly related to wine composition. As described before wine components alone or combined affect sensorial perception of wine and thus overall wine quality.

Volatile composition of the wine affecting the aroma is a determinant factor in wine quality, and ethanol is one of the main components affecting aroma perception.

2.1.8 Chemical and sensorial effect of ethanol

The three more abundant by-products of alcoholic fermentation in wines are ethanol, carbon dioxide and glycerol, respectively (Pretorius 2000). Glycerol, like ethanol, plays an important role in sensory characteristics of wine, particularly smoothness and overall body (Nobel and Bursick 1984, Pretorius 2000). Ethanol being the most abundant volatile compound has a strong impact on the quality of the wine and thus on the organoleptic properties of aroma an taste, affecting acidity, astringency, sweetness, flavour intensity, textural properties and volatility of aroma compounds (Mermelstein 2000, Whiton and Zoecklein 2000, Hartmann et al. 2002, and Varela et al. 2012) (Table I).

Wine is a complex mixture of compounds that interact between them altering their perception. Aroma and mouthfeel differences have been described to be affected by compound interaction. Several authors have investigated the effect of ethanol on wine aroma using analytical and sensorial approaches. Analytical data obtained by measuring the activity and partition coefficient of volatile compounds (i.e., equilibrium distribution of a compound between the sample phase and the gas phase. Partitioning of volatile substances between the liquid and gas phases are highly determined by the compound volatility and solubility (Pozo-Bayón et al. 2009)) using static headspace and gas chromatography techniques, found significant differences between artificial wines and water for many volatile compounds (e.g., isoamyl acetate, ethyl hexanoate, n-hexanol and β-ionone). However, not many studies have evaluated the effect of ethanol on volatile delivery, which in terms of wine aroma is the most interesting (Tasachaki et al. 2005, 2008).

Sensorial studies on the ethanol effect on the the perception of wine aroma are more frequent. Using both model and real wines various authors tested the effect of different ethanol contents on panellists' perception of different aroma characteristics and also the interaction with other wine compounds (e.g. tannins, sugar, etc.). Ethanol has a suppression effect on aroma, requiring higher odour thresholds for aroma compound detection (Grosch 2001). In sensorial investigations using model wines, decreasing ethanol content impacted the aroma perception by increasing an overall fruity and floral intensity (Guth 1998, Escudero et al. 2007); at 0% ethanol the aroma descriptor was an apple-like one that decreased in intensity until it being no longer detected at 14.5% ethanol (Escudero et al. 2007).

Increasing ethanol concentration (from 10-12% to 14.5-17.2%), in Malbec wines, proved not only to decrease aroma intensity but also to change the perceived aroma from fruity to herbaceous (Goldner et al. 2009). Villamor et al. (2013a) while investigating combined effects of ethanol, tannins and fructose, reported that higher concentrations of these three wine components cause a strong reduction in aroma perception measured through headspace concentrations. For all odourants studied the detection thresholds increased as ethanol, tannins and fructose increased.

But ethanol as also known effects in mouthfeel and flavour perception. Investigating how different levels of ethanol ethanol (4–24 vol.%) affected sweetness, bitterness acidity and saltiness Martin and Pangborn (1970) used different model solutions and reported ethanol to enhance the sweetness of sucrose and the bitterness of quinine whereas it reduced acid and saltiness perception of citric acid and NaCl solutions, respectively. But the sourness perception diminished with increasing ethanol (from 8 to 13%) in previously dealcoholized wines (Fischer and Noble 1994). In red wines lowering ethanol level resulted in a longer finish for floral and coconut flavours (Baker and Ross 2014b). High levels of ethanol in white wines were reported to enhance the perception of hotness but for body and viscosity the results were not significant (Gawel et al. 2007), contrarily to what Pickering et al. (1998) had showed also in white wines in which maximum viscosity was perceived for the lowest ethanol level (3%).

Wine finish, defined as a lingering flavour, taste and mouthfeel sensored after swallowing or expectorating wine (Jackson 2009), is very relevant to the consumer final opinion about wine quality as it is the last impression one keeps. It is considered a temporal attribute and measured as so in time duration (usually in seconds). Studies investigating this quality attribute remarked that flavour finish, in average longer for red wines, varies considerably depending on the flavour evaluated. For example, fruity notes were reported to finish earlier than floral notes in white wines evaluated by a trained panel (Goodstein 2011). In red wine, not only a longer finish is related with a better quality and increased acceptance (Baker and Ross 2014a), but also the content of ethanol changes finish perception. The same authors showed that increasing ethanol contents (9% to 14%) increased the flavour finish perception for bell pepper (3-isobutyl-2-methoxypyrazine), floral (2-phenylethanol), and coconut (oak lactone) flavours (Baker and Ross 2014a,b).

Tannin concentration also positively influenced finish perception for bell pepper flavour but not for the other two flavours. The effect of ethanol and tannin concentrations on flavour and aroma is not surprising and has been described in numerous studies (e.g., see above, Robinson et al., 2009; Secor, 2012; Villamor et al. 2013a,b). Higher tannin and low ethanol concentrations increased the perceived aroma. Moreover, bitterness intensity and persistence was longer when ethanol content was higher but not when catechin and tannic acids were added (Fischer and Noble 1994). Contrarily, in the same study, astringency was not significantly affected by ethanol variation nor enhanced salivary production (a reaction related to an increase of astringency in mouth). Jones et al. (2008) studied the interaction effect of wine proteins, ethanol and glycerol, reporting altered aroma intensity at lower volatile concentrations. In another study investigating astringency and bitterness perception, it was shown that the interaction among ethanol, sugar, organic acids and phenolic compounds elicit both sensations (Noble 1999). Villamor et al. (2013a) in the same study model red wines with different combinations of ethanol, tannins and fructose were given to taste to a panel, but no significant impact of the interaction effect.

TABLE I. THE EFFECT OF ETHANOL ON AROMA AND FLAVOUR PERCEPTION

	Ethanol Effect
sweetness	↑
bitterness	↑
acidity	↓
saltiness	↓
hotness	↑
viscosity	↑
sourness	↓

	Ethanol Effect
fullness	↑
fruity/floral	↓

Interestingly, Boulton (2001) showed an unexpected effect of ethanol on copigmentation phenomena, reporting that ethanol decreases anthocyanin copigmentation, as the presence of such organic solvents weakens the intramolecular hydrophobic interaction and inhibits complex's formation of self-association (anthocyanins molecules are copigments themselves) (González-Manzano et al. 2008). Less ethanol implies a greater proportion of flavilium form of anthocyanins, leading to an increase of the colour intensity and a decrease in the hue, plus an increase in the blue tonality characteristics of the younger wines (Bogianchini et al. 2011; He et al. 2012).

2.1.9 Alcohol content increase

Climate change and vinification processes can be pointed out as the main causes of the observed increment in alcohol content of the wines. Climate change mathematical models preview a shift and expansion towards the higher latitudes of the north of Europe of the viticole regions, with a degradation of the southern Europe viticole regions that tend to become to hot and dry for production of quality wine (Jones 2005, Jones et al. 2007).

Many vine phenological studies have shown an anticipation of the harvest dates due to an early ripening and optimal maturation of the grapes (Bock et al. 2011, de Orduña 2010, Schultz and Jones 2010, Fraga et al. 2013). In France and in Spain, harvest dates have been anticipated by 2-3 weeks in some regions. Jones et al. (2007) has shown that in the Galicia Community and Valladolid province, average temperatures raised 0.8ºC and 1.2ºC, respectively, and more significantly night temperatures, and simultaneously harvest dates were shifted between 6-15 days before they used to be.

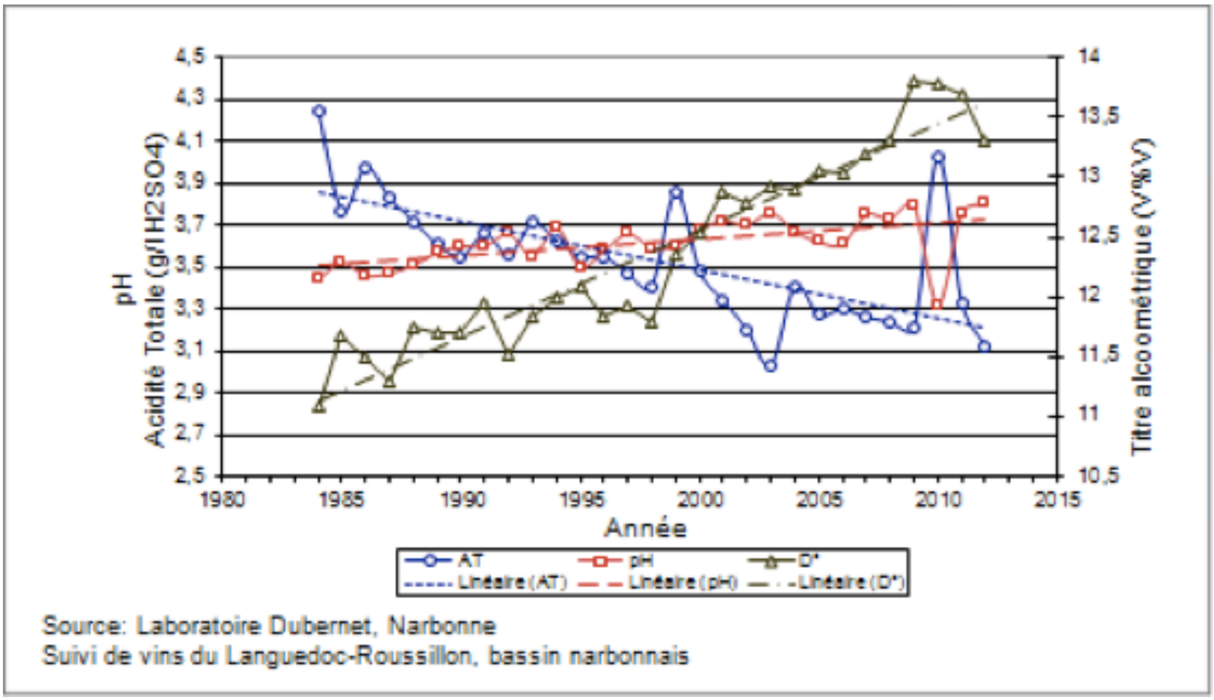


FIGURE 2. EVOLUTION OF THE ANALYTICAL COMPOSITION OF WINES (TOTAL ACIDITY PH AND ALCOHOL) FROM 1984 TO 2015 (SOURCE: FOR THE WINES IN THE NARBONNAIS BAY IN LANGUEDOC-ROUSSILLON, FOLLOWED BY LABORATOIRE DUBERNET, NARBONNE)

These changes in the climate but also the evolution of winemaking decisions and processes has lead to the production of higher alcohol content wines (fig. 2). Grapes are harvested at their optimal maturation point but with higher sugar contents and lower acidity (lost by natural degradation of, especially, the malic acid during plant cell respiration).

The quality of the wines may be in risk if the raw product - the grapes - is not itself in balance. Higher alcohol, lower acidity (higher pH values), worse ageing capacity (less microbial stability, poor chemical composition) and overall reduced wine quality (herbaceous flavours, colour unbalance, bitter tannins, astringency, etc.).

2.2 Alcohol management: different technologies to reduce alcohol

Alcohol removal technologies have been available since, at least, the early 1900s. In Rüdesheim am Rhein, in the Rheingau area, in Germany, there is a winery Carl Jung that has been producing dealcoholized wine since 1908, for over 100 years (JungWines, 2015). They have, in fact, patented their process of alcohol extraction from wine, but since then technologies have evolved and deeply changed and improved in many ways. The challenge remains, however, the same that is to produce a wine that looks, smells and tastes equally pleasant as a “normal” untreated wine, maintaining the main organoleptical characteristics after the alcohol removal.

Academics and industry have been working in developing new technologies and improving the already existent in an attempt to reduce alcohol content without compromising the quality of the wine, but also and more importantly for the industry, in such way that production costs do not affect competitiveness (national and international). Studies and industrial experience has shown that, in general, thermal methods have more severe effects in the wine, and thus the trend today is to use alternative methods like the membrane based ones.

There is a great panoply of different techniques available that can be applied already in the vineyard or later on in the cellar during the vinification process (Table II). This variety of methods enables its application directly to the base product - the grapes and the must - through reduction of sugar contents (e.g., use of grapes with lower sugar content and/or use of specific enzymes); to the intermediate product as it is being processed either during alcoholic fermentation and malolactic fermentation (e.g., via thermal processes or membrane filtration); or lastly, to the final product - the wine - keeping in mind that at this point the product to be treated is more sensible (Teissedre 2013).

TABLE II. TECHNOLOGIES FOR REDUCING ETHANOL CONCENTRATION IN WINE AND FERMENTED BEVERAGES (ADAPTED FROM PICKERING 2000 AND SCHMIDTKE ET AL. 2012)

Stage of wine production	Acts on	Principle	Technology/Technique
Prefermentation	Sugar	Reduction of fermentable sugars	Crop management Early fruit harvest Juice dilution Freeze concentration and fractionation Use of enzymes (e.g. Glucose oxidase)

During sugar fermentation	Reduction of alcohol production	Selected/modified yeast strains Arrested fermentation Use of metabolic inhibitors (e.g. furfural)
Postfermentation ethanol	Membrane separation	Reverse osmosis Pervaporation Osmotic distillation
	Non-membrane extraction - thermal and extraction processes,	Solvent extraction, supercritical carbon dioxide Ion exchange (resins; silica gel) Spinning cone column
	Winemaker decision	Blending Dilution of wine

2.2.1 In the vineyard

Sugar content of the grapes is a major determinant of alcohol level in the wine. As grapes ripen sugar levels increase and further on concentration rises due to dehydration. Vineyard management and practises to control grape maturation are crucial.

Vineyard management

Increasing yield may be the most evident strategy to reduce sugar content in grapes, and can be achieved by increasing the bud load, lowering cluster thinning, choosing a vigorous rootstock and minimal pruning system. This strategy is not without risks to wine quality as fruit quality may suffer detrimental consequences.

Managing canopy beyond disease and pest control has shown some effects in grape quality, namely affecting the rate of sugar accumulation that is greatly correlated to the ratio of leaf area to fruit weight (LA/FW, Kliewer and Dokoozlian 2005, Stoll et al. 2010). High ratios of LA/FW lead to high concentrations of sugar by the time grapes are mature, flavour and phenol content wise. The contrary, lowering the ratio LA/FW has been shown to reduce sugar accumulation in rapes (Kliewer and Dokoozlian 2005). Basal leaf removal undertaken at the veraison period negatively influences sugar accumulation enhancing phenol production by enzymatic activity (Korkutal et al. 2013, Di Profio et al. 2011). Many trials have been done experimenting with different defoliation patterns at different times from veraison to to a few weeks after, and the results varied from lowering sugar content to affecting pH/acidity but also anthocyanin and polyphenol concentration (e.g., Stoll et al. 2010, Balda and Martínez de Toda 2011, Lanari *et al*. 2013).

Carefully choosing the time to defoliate the vine can seriously impact the sugar levels, and thus the alcohol content in the wine. Every vineyard is different and these practices should be tested, but studies report that reducing leaf area after fruitset demonstrated to lead to a better synchronisation of sugar and flavour/phenolic ripening relation, and thus better management of sugar and alcohol content.

Other strategy maybe to reduce shoot vigour combined with obtaining small berries and clusters rich in phenols. This however requires a proper irrigation management, pruning intensity and new *V. vinifera* genotypes (Clingeleffer 2007) that possibilitate good quality grapes at a lower maturity stage concerning sugar content.

Anticipating the harvest date, can be more detrimental than beneficial, as the lack of maturity of the grapes will affect the phenolic compounds, giving place to the extraction of more aggressive tannins (more astringent) and a stronger herbaceous character, resulting, probably, in to an unbalanced wine. One compromise here to take advantage of these still “green” fruits is to do a “double harvest”. The first harvest would coincide with this yet green stage and the second one at the “normal” maturity. The final wines resulting from a blend of these two harvests, as has been tested, show a lower alcohol content and pH and a higher total acidity. Taste and quality wise when compared to those from traditional winemaking they could be distinguished (Kontoudakis *et al.* 2011; Balda and Martinez de Toda 2013).

Managing photosynthesis and maturation

Controlling photosynthetic rates can directly influence the sugar accumulation, as well as grape ripening and quality. Strategies like the application of shade nets over the vine or the application of anti-transpirant sprays have shown to influence the CO_2 uptake which a direct impact on grape quality with a significant reduction of sugar accumulation (Palliotti et al. 2010, Tittmann et al. 2013).

Hormone treatments - use of plant growth regulators - may be also be used to delay maturation processes. Though not yet widely explore, it is known that abscisic acid and ethylene influence colour development and the levels of auxin suffer a reduction when the fruit ripening begins. Böttcher and colleagues (2011) applied auxin pre-veraison in Shiraz vines and delayed grape ripening in terms of sugar content and anthocyanin content. It is thus promising this type of strategy.

2.2.2 In the cellar

When viticulture is not enough or not an option, acting in the cellar may be the alternative. Technical strategies have long been present and research and industry work together to improve the different methods, making them as good as possible and also cost competitive.

Alcohol reduction methods can be applied at different moments of the winemaking process (table II) with different approaches. Pre-fermentation applications look forward to reduce sugar content in the must whereas during and post-fermentation applications act directly on the alcohol level. After the wine has been produce alcohol can still be removed, and in the wine industry this is the strategy mostly used.

Microbiological and enzymatic methods

Choosing the best yeast strain to carry out alcoholic fermentation with a specific must is more and more a determinant decision to be taken by the winemaker. From spontaneous fermentation to a whole wide range of commercially available yeast strains, one can choose potentially one specific type for every different kind of wines, guaranteeing fermentation reliability and predictability. Choosing strains with lower ethanol yields is one strategy to reduce alcohol content in wine. Despite the high strain phenotypic diversity of wild *Saccharomyces cerevisae*, the difference in percentage of ethanol production during fermentation metabolism is not exceeding 0.5% to 1% (Jenson 1997; Dequin 2007; Varela et al. 2008). Genetically engineered *S. cerevisae* maybe an alternative, altering metabolism from producing ethanol to other metabolites that allow to maintain wine quality (e.g., Bartowsky et al. 1997; Malherbe et al. 2003), Research has focused on finding yeast alternatives to *S. cerevisae*, among non-*Saccharomyces* yeasts that can metabolise sugar either without producing ethanol or producing in very small quantities. Naturally present in the must (originated from grapes or the cellar itself) these non-*Saccharomyces* yeasts are usually not capable of completing alcoholic fermentation, and thus strategies like applying sequential inoculation with non-*Saccharomyces* followed by *Saccharomyces* yeasts (Contreras et al. 2014, Quirós et al. 2014) or co-inoculation are now being studied and tested. One other question that is raised when relying on non-*Saccharomyces* yeasts is that the by-products of sugar metabolism differ from those produced by *S. cerevisae* altering, potentially, wine composition, flavour and aroma (Ciani and Maccarelli 1998; Magyar and Toth 2011).

Pichia and *Willopsis* yeasts have demonstrated to be a potential alternative to *S. cerevisae,* producing low alcohol wines with acceptable palate and quality when compared to wines produced with *S. cerevisae* (Erten and Campbell 2001). Other studies have reported a reduction in alcohol content with no detrimental effects to the wine quality by using non-*Saccharomyces* yeasts and *S. cerevisae* strains in co-inoculation or sequential inoculation with up to 1.6% less ethanol compared to the ethanol concentration achieved with a single *S. cerevisae* inoculation (e.g., Sadoudi et al. 2012, Contreras et al 2014).

Most if the recombinant strains were targeted to increase glycerol production at the expense of ethanol (e.g., Michnick et al. 1997). Though not available commercially, altering *Saccharomyces* sugar metabolism to produce other by-products rather than ethanol has proved successful. By over expressing *GPD1* or *GDP2* gene, coding for glycerol 3-phosphate dehydrogenase (GPDH), yeast strains metabolism is redirected to produce more glycerol and less ethanol, circa 1-1.5% less (Michnick et al. 1997, Remize et al. 1999, Nevoigt et al. 2002, Nevoigt 2008). Recently it was developed a *S. cerevisae* strain producing GOX by integrating into the yeast genome the *Aspergillus niger GOX* genes. The wine yeast transformant (modified yeast strain) was tested under red and with experimental winemaking conditions with successful and promising results. Alcohol reduction using the GOX-*Saccharomyces* resulted in a final wine with less 1.8-2% less ethanol (Malherbe et al. 2003, Malherbe 2010).

So far, however, to my knowledge no genetic engineered strains have led to desirable oenological results. More studies are needed and also so far genetically modified yeast strains are not permitted by OIV and EU in winemaking.

Glucose Oxidase (GOX)

Grape must is treated with glucose oxidase (GOX, EC 1.1.3.4) enzyme. GOX is an aerobic dehydrogenase that catalyses the oxidation of glucose into gluconolactone and hydrogen peroxide in the presence of molecular oxygen, which is then non-enzymatically hydrolysed to gluconic acid (Villettaz 1986). The content of glucose available to be metabolised by yeast cells is reduced, and consequently leads to a lower alcohol content in the wine. This method though successful is quite labour intensive and requires some expertise from the winemaker as the enzyme needs to be added to the must as a specific time. Optimal conditions for the enzyme require the presence of molecular oxygen which can cause the oxidation of other compounds and negatively affect organoleptical characteristics of the wine. It also requires relatively high pH, between 5.5-6, making it necessary to deacidify the must previous to GOX application, normally with calcium carbonate. Finally, the production of gluconic acid increases the total acidity of the many and can lead an unbalanced wine.

Glucose conversion by GOX in must has been optimised (Pickering et al. 1998) showing that the low pH of the must in the main limiting factor in the process (Fig. 3). In commercial GOX usually the preparations have a second enzyme to degrade the hydrogen peroxide that is formed.

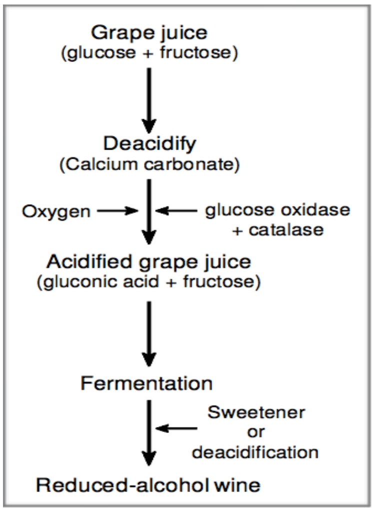


FIGURE 3. GOX UTILISATION IN PRODUCING REDUCED ALCOHOL WHITE WINE (FROM PICKERING ET AL. 2000).

GOX use is presently limited to white grape varieties must, as clarification prior to enzyme reaction must occur before yeast inoculation for alcoholic fermentation to start (Schmidtke et al. 2012). Pickering et al. (1999a,b,c) tested the use of GOX to produce reduced alcohol wines and evaluated the composition of the resulting wines as well as their sensorial characteristics. Chemical composition compared to untreated wines showed some differences. The potential to reduce final alcohol content in the wine was high, with up to 40% reduction in the ethanol yield. The high levels of gluconic acid produced rendered wines out of balance, and also there was an increase in esters and fatty acids. One other issue is the formation of carbonyl compounds promoting high sulphur dioxide binding and consequently requiring higher total concentrations of sulphur dioxide to achieve microbial stability in GOX treated wines. The presence of oxygen, as mentioned before, also proved to increase premature browning with increased flavonoid production. Moreover the wines had a deeper colour.

As predictable from the chemical analysis, sensorially the wines were more acidic (due to the gluconic acid), and the taste and appearance attributes were significantly modified. However the aroma appeared to be relatively unaffected.

Fermentation arrest

A simple strategy to produce wine with a low alcohol content is just to stop the alcoholic fermentation whenever it has achieved a desirable alcohol level. This is not without risks because the concentration of residual sugar will be, naturally, high, the aroma and flavour will also be affected as the yeast may have not the change to produce all the desired metabolites resulting from fermentation metabolism. High levels of residual sugar are prone to get more easily microbial spoiled requiring higher levels of SO_2 or any other wine preservative.

2.2.3 Physical Processes: Thermal methods

Vacuum Distillation

Vacuum distillation involves distilling or concentrating the wine under vacuum conditions by means of a continuous flow, recovering both the distillate and the dealcoholised and partially dearomatised wine. The lower the temperature used in the system the less flavour and aroma degradation occurs to the wine. Also if it lasts too long there might be formation of hydroxy-methyl-furfural, as a result of overheating of pentoses and hexoses when in acidic medium as it is wine (it is very common in sweet Madeira wines that are aged in heating circumstances, see for more detail Pereira et al. 2011).

In wine industry the use of high-vacuum and low temperature treatments is less severe than distillation at atmospheric pressure that implies submitting the wine to temperatures close to 100ºC for 20-30 minutes. Alcohol is removed but along with a lot of volatile aromas and with high chances of degrading the quality of the final product. As in many other treatments, there is a loss of volume that depends on the amount of alcohol to be removed, and increases the more the alcohol content to be removed.

Aguera et al. (2010a) extracted ethanol during wine fermentation (approximately 2%) by vacuum distillation and stripping by CO_2. They concluded that this procedure had little effect on wine characteristics, as by-products of yeast metabolism of sensory importance were subsequently resynthesised. In the second part of fermentation (after partial dealcoholisation), the synthesis of higher alcohols holds higher yields. Acetate, though partially eliminated by distillation, was *de novo* synthesised, as it is needed for acetylCoA and NADPH metabolisms for yeast growth and maintenance (Saint-Prix et al. 2004).

Spinning cone column

The spinning cone column (SCC) is a world wide industrially used evaporation process, developed by an Australian company. It is a gas-liquid contact device that consists of a vertical countercurrent flow system containing a succession of alternate rotating and stationery metal cones with surface coated with a thin film of liquid. Alcohol removal is a two-step distillation process. In the first step, as the wine passes the column the aromatic volatile fraction is removed at high vacuum conditions (0.04 atm), low temperatures between 26-28ºC and collected together with an ethanol stream representing approximately 1% of the original wine volume. The second step is the separation of the ethanol from the dearomatised wine, at higher temperatures (~36-38ºC). After the ethanol removal, the aromatic fraction is added back to the dealcoholised and dearomatised wine, resulting in a long and expensive operation (Belisario-Sánchez et al 2009; Schmidtke et al. 2012). In the winemaking industry SCC is not only used for alcohol removal but also in recovery of aromas, removal of sulphur dioxide and concentration of grape juice.

Supercritical solvent extraction with CO_2

The principle behind the process is to compress a gas at temperatures above its critical point resulting in the formation of a supercritical fluid with high solvent capacity, that allows for liquid separation or extraction. Carbon dioxide is commonly used in food and beverage industry for supercritical extraction as it, with the advantage that in the wine industry does not pose any legal issues and is a really good gas for extraction of ethanol (Marignetti et al. 1992). The volatile fraction containing the alcohol and aroma compounds is then subjected to supercritical extraction under a pressure of 80-100 bars. The aroma fraction is then recovered from the column head and the ethanol-water component is drained away as a liquid from the bottom of the column (Schmidtke et al. 2012).

Freeze concentration

Mostly used to remove water from grape juice, traditionally the juice or wine was placed on a tank equipped with a heat-exchanger leading to the formation of a block of ice; the juice or wine was then removed and the water (with alcohol) drained away as the ice thawed - this process would be repeated until achieved the objective. More modern equipment relies on an automatised system with a continuous feed of product through a crystalliser to a recrystalliser. Ice crystals form and grow in the recrystalliser until they achieve a certain size, and then they are transferred to a wash column where concentrate and water is removed (Wollan 2010). Afterwards the ethanol is removed from the residual liquid by vacuum distillation or by cooling the wine.

2.2.4 Physical processes: membrane methods

Membrane methods rely on semi-permeable barriers or membranes that allow to separate ethanol from wine (or other fermented beverages). They can organised into two main groups according to the process driving force: either a concentration gradient or a pressure gradient. In the first group, it can be included dialysis, whereas in the second group, we can have reverse osmosis.

Ultrafiltration and Nanofiltration

Nanofiltration (NF) is a separation method based in membrane under pressure, with and intermediate capacity between ultrafiltration (UF) and reverse osmosis.

Aguera et al (2010b) tested a pilot machine REDUX® developed by Bucher-Vaslin in 2004, based on membrane separation methods that combines ultrafiltration (0.01-0.1µm pores) with nanofiltration (0.001-0.01µm pores) under a pressure of 6 bars and 70 bars, respectively, to remove sugars from must (Cottereau et al. 2006, 2011). Ultrafiltration is here used to separate must macromolecules before the concentration step (very evident in the colour). The resulting retentate from UF very rich in macromolecules is reblended with the original must. In the second step, the permeate (impoverished in macromolecules) from UF is concentrated by NF, leading to a reduction of around 34 g/L of sugar, obtaining a hyper-concentrated of sugar of about 400g/L (Aguera et al. 2010b) (Fig. 4).

The must with reduced sugar content was then subjected to alcoholic fermentation and posterior chemical analysis shown that the resulting wine with lower alcohol content had volatile components present in slightly lower concentrations but was richer in dry extract than in the control wine, and the flavour and aroma were not significantly different from the untreated wine. Also they reported a great loss of must volume during the treatment (around 7% per probable degree removed) which is an inconvenient to the winemaking.

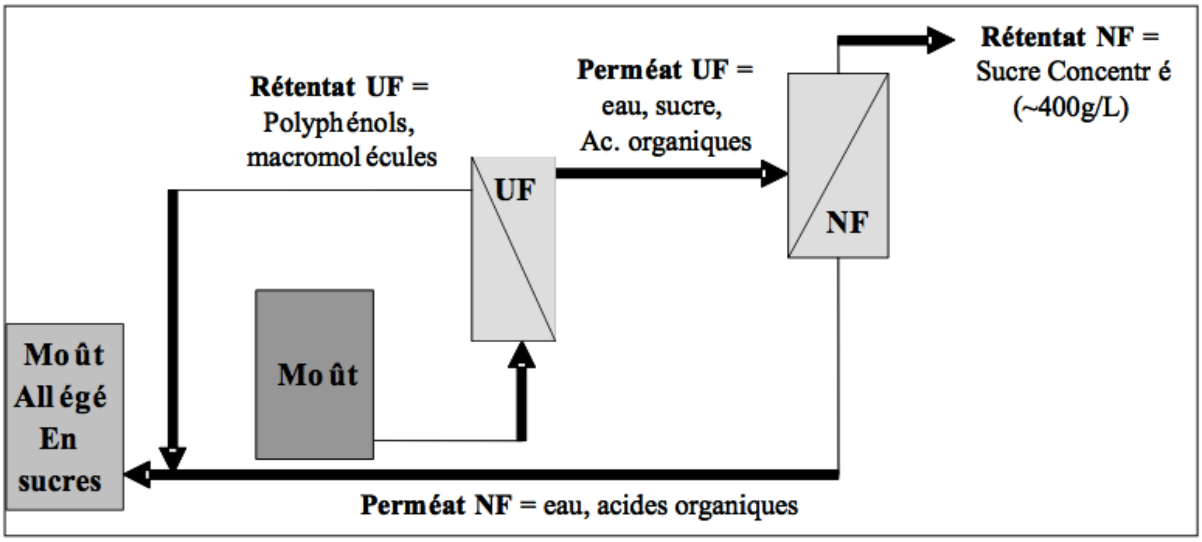


FIGURE 4. SCHEME TO REDUCE SUGAR CONTENT IN MUST BEFORE FERMENTATION BY COMBINING ULTRAFILTRATION AND NANOFILTRATION (ADAPTED FROM AGUERA ET AL. 2010B).

Currently, the Redux® method is only based in NF (no UF applied to the must) to avoid higher losses of must volume, but at the same time obtaining wines as good as before (Cottereau et al. 2006, 2011).

Reverse osmosis

In this membrane method as mentioned before the driving force is a pressure gradient; a sufficient high pressure is applied on the solution with higher concentration, forcing the solvent to move out of this solution across the membrane, contrarily to what would happen in a process of osmosis. The wine is filtered through a nonporous selective membrane only permeable to water and alcohol but not to most of the other dissolved components, under high operation pressures (60-80 bars), with a potential severe impact on the organoleptic properties of the wine (Gonçalves et al. 2013). Some aroma compounds like esters and aldehydes; organic acids and potassium can also diffuse through the membrane (Schobinger et al. 1986). This technique leads to a dealcoholised but concentrated wine (retentate) and a solution of water and ethanol (permeate).

Posteriorly, the method requires a re-mixture of the retentate for further diluting with water filtered from the wine itself. This remixture can create a legal issue in countries where adding water is not allowed (such as the EU members). To overcome this legal obstacle either low sugar grape juice is added or a second RO unit is used in parallel, being equipped with an ethanol impermeable membrane and having the filtrate from the 2nd unit redirected to the wine supplying tank (feed supply) of the ethanol permeable unit (Bui et al. 1986). One other option used in the wine industry is to thermal distillate the permeate resulting from the RO process and redirecting the water component into the wine tank in a closed-loop (Smith 1996). The ethanol is separated and condensed reaching values close to 90% v/v.

Wines with 15% v/v can be treated with RO to produce alcohol contents as low as 0.5% v/v, but the greater the reduction the greater the effect on the wine quality (Bes et al. 2010). In comparison with other methods to remove alcohol from wine, RO treated wines usually show flavour and aroma profiles comparable to regular untreated wines (Gil et al. 2013).

This technique is also use in the wine industry to remove colour and flavours, to concentrate must (as an alternative to chaptalisation), to stabilise wine against tartaric precipitation, to deacidify and more specifically to reduce volatile acidity (Smith 2002).

Osmotic distillation (or evaporative perstraction)

OD is a membrane based technology in which wine (feed phase) circulates through a hydrophobic hollow fibre membrane contactor and water (strip phase) flows through the other side of the membrane inside the hollow fibre contactor (Diban et al. 2008). The pressure difference of the volatile compounds between both phases creates the driving force, and makes the exchange happen; ethanol is removed from wine and added to the water. The process is based on the principle that ethanol, as a volatile component, as an important vapour pressure, leading to the evaporation from the liquid into the gas phases in the porous matrix of the hydrophobic membrane.

Aside from the easiness of the process, this technology brings many advantages in comparison to other membrane and thermal methods: the process can be conducted at room temperature and atmospheric pressure (less energy needed, reduced loss of volatile compounds, internal pumps produce the flow and pressure difference between feed and strip is the driving force), produces no hazardous by-products (extractants) as water is normally used as strip phase (though, in some countries of EC it is required to have a distillation permit to run it).

Analytical and sensorial analyses made to partially dealcoholised (up to 2%v/v) wine by OD have shown little change on the sensory properties and insignificant alterations in the main phenolic compounds, the colour and the volatile acidity of different red wine varieties (Diban et al. 2008, Gambuti et al. 2011, Lisanti et al. 2012, Diban et al. 2013).

2.2.5 Stabilisation, conservation and preservation of alcohol reduced wine

Partially or de-alcoholised wines maybe more prone to microbial spoilage due to the reduced content of ethanol that itself acts as a antimicrobial agent. In this sense, reducing levels of ethanol (as well as increasing levels of residual sugar) may lead to an increase in preservatives usage namely sulphur dioxide the most common one in the wine industry. Preservation techniques used in fruit juices and beer may bring important insight to the wine industry. The search for new preservation techniques that enhance product quality (or at least to do not degrade it) with the minimal organoleptic impact are the new trend of the fruit juice industry (review in Aneja et al. 2014). Processes using non-thermal methods and natural antimicrobial compounds and ideally eco and health friendly, urge to be found (Table III).

TABLE III. DIFFERENT STRATEGIES TO LIMIT OR HALT MICROBIAL SPOILAGE IN WINE (ADAPTED FROM ZOECKLEIN ET AL. 2005 AND BARTOWSKY 2009).

	Antimicrobial Agent	**Mechanism of Action**
Most common	**Sulphur Dioxide**	Microbial development inhibition
	Filtration	Physical removal
Chemical	**Dimethyl dicarbonate (DMDC)**	Enzymatic inhibition

	Antimicrobial Agent	Mechanism of Action
	Potassium Sorbate	Growth inhibition
Natural products	**Lysozyme**	Cell lysis by cell wall disruption
	Bacteriocins	Cell lysis by cell wall disturbance
	Chitosan	Cell membrane disruption
New technologies	**Ultrahigh pressure**	Cytoplasmatic membrane damage and enzymatic inhibition
	Ultrasound	Cell membrane disturbance
	UV radiation	DNA damage
	Pulsed electric fields	Dielectrical breqkdown of cell membranes

Chemical preservatives such as sodium benzoate and potassium sorbate are often used to prevent microbial spoilage of fruit juices (Tribst et al. 2009) but also in the wine industry (Ough and Ingraham 1960, Zoecklein et al. 2005). The use of these two preservatives has, however some drawbacks. Potassium sorbate is not an effective yeast killer, it only prevents them from growing and being active and has no effect in lactic acid (LAC) and acetic acid bacteria (AAC). In the presence of ethanol, sorbic acid is reduced to form ethyl sorbate, known to bring pineapple and celery notes to the wine. Also, if lactic acid bacteria are present they will metabolise sorbic acid producing a strong undesirable odour of geranium leaves. Sodium benzoate cannot be used according to the EU directives (EU/2/95 directive).

DMDC is also used to protect wine, but contrary to the other mentioned products it as a momentaneous effect not useful for a long time conservation, as it does not remain active for long. It is a chemical inhibitor that acts by enzymatic inactivation of the cells, leading to cell death by metabolic failure (Daudt and Ough 1980). When added to the wine it rapidly hydrolyses to methanol and carbon dioxide, usually not affecting organoleptic properties. Recent studies have suggested that it is not fully efficient against bacterial spoilage (Costa et al. 2008).

But as consumers demand lower alcohol content in wine for health issues they also demand natural origin preservatives other than SO_2 or potassium sorbate. Natural antimicrobials have been tested but to my knowledge none succeed to substitute SO_2. Lysozyme is widely used against LAC and AAC, as it destroys the bacterial peptydoglican cell wall of gram positive bacteria (gram negative only contains 5-10% peptidoglycan in the cell wall) (Raybaudi-Massilia et al. 2009). But it is not effective against eukaryotic cells such as *Brettanomyces/Dekkera*, due to differences in cell wall structural components (McKenzie and White 1991). Though no studies have referred any alteration in the aroma of wine, lysosymes binds to tannins and polyphenols (in red wines) resulting in a decrease of colour and may form wine haze (Bartowsky et al. 2004 and Bartowsky 2009). Bacteriocins are small polypeptides produced by some LAB, that inhibit other bacterial species by inducing cell lysis (Bruno et al. 1992).

Chitosan is also effective against microbial spoilage, its positive charged amino group at C2 can bind with negative charged groups of the cell surface such as lipopolysaccharides and proteins, disrupting the integrity of the outer membrane. In addition, essential oils part of a group of terpenoids and sesquiterpenes, act not specifically but due to their hydrophobic characteristics, disrupting the lipid structure of the cell membrane and mitochondria rendering them more permeable.

Another strategy is the use of oenological products such as phenolic compounds (e.g. resveratrol, hydroxytyrosol, quercetin, hydroxycinnamic and hydroxybenzoic acids) with demonstrated antimicrobial activity by inhibiting growth (Papadopoulo et al. 2005, Vaquero et al. 2007; García-Ruiz et al. 2008).

For stabilisation of low alcohol wines it is often used ascorbic acid which can lead to higher anti-oxidant activity in such wines (Bogianchini et al. 2011). The use of ascorbic acid, on one hand has no allergenic effect has it does SO2 but on the other hand, these authors showed that phenolic composition is not stable over time. Caffeic acid was reported to disappear and caftaric and ellagic acid concentrations suffered significant reductions (25 and 43% respectively). Taste wise no alterations were mentioned.

2.3 Legal issues: What is wine?

Wine by definition is a grape derived beverage with a certain content of alcohol as it results from the fermentation of sugar into alcohol by yeast. According to the OIV and EC definition (OIV 18/73) wine is “the beverage resulting exclusively from the partial or complete alcoholic fermentation of fresh grapes, whether crushed or not, or of grape must. Its actual alcohol content shall not be less than 8.5% (v/v)”. The concentration of alcohol varies among the different wine definitions in the different countries with, for example, a minimum of 4.5% (v/v) in Australia (ComLaw 2014), between 7-24% (v/v) in the USA (FAA 2015).

These definitions exclude from the wine group all beverages resulting from fermented grapes with lower alcohol concentrations. Hence causing some confusion when naming the so-called “dealcoholised” and “low- or reduced-alcohol wine” as such is not legally accepted as wine. There are, however, some exceptions allowed by the OIV for particular wines with a reduced alcohol content as low as 7% (v/v) due to “climate, soil, vine variety, special qualitative factors or traditions specific to certain vineyards” in some regions.

Partial dealcoholisation of wine is allowed since 2009 (Resolution 10/2004, EU nº 606 from July 2009), as far as it does not surpass a maximal reduction of 2% relative to the original alcohol content (OIV-ECO 433-2012). The subtractive techniques to be used for dealcoholisation are described in the appendix 10 from regulation. So far, the use of extractive techniques to reduce sugar content in the must are forbidden except under special authorisations, and the same rule applies to alcohol reductions superior to 2%.

Also to do partial dealcoholisation of wine several requirements are to be followed:

- the reduction of alcohol content cannot surpass 2% and the resulting alcohol content of the final product must be within the legal range for wine (not less than 8.5%);
- treated wines must not present any organoleptic faults and must be apt for human consumption;
- if any of the products used to produce the wine has been subjected to any processes to increase the alcohol content, the wine cannot be posteriorly dealcoholised;

- any dealcoholisation (partial or not) treatment must be registered and the authorities informed.

Despite the definition of wine and its alcoholic content, different laws apply to the technology used to reduce the alcohol content in different countries and economic trade regions. The different technologies can be applied both prior or after alcoholic fermentation, from the vineyard to the winery.

OIV as mentioned before regulates the technologies and their applications in must and wines. Regulation CE 606/2009, allowed for partial dealcoholisation of wine by means of separative physical techniques. As from June 2010, OIV approved the use of membrane based methods to must and wine, regulations 373A/2010 and 373B/2010, respectively. This update is an attempt to ameliorate the quality of dealcoholised wines, having as clear objectives:

- elaboration of wine with balanced organoleptical properties;
- compensate for adverse climate conditions, for climate change consequences and correct particular organoleptic faults;
- following consumer demands, increase the number of available possible techniques for alcohol removal.

The following membrane based methods can be used individually or in combination: microfiltration, ultrafiltration, nanofiltration, membrane contactors, reverse osmosis and electromembranes, always in accordance with the International Oenological Code and the International Code for Oenological Practices (OIV 2012). These methods can be used both in musts and wines to reduce the concentration of certain organic acids, to adjust total acidity and pH and for partial dehydration. In musts sugar content can also be reduced and in wines alone these methods can be applied for tartaric stabilisation, partial dealcoholisation and reduction of volatile acidity.

As recently as 2012, OIV came up with new definitions and procedures on alcohol reduction, in response to the market demands and new advances in technology.

Accordingly, wines can be submitted to dealcoholisation (OIV OENO 394A-2012) or have their alcohol content corrected (OIV OENO 394B-2012) by removing excessive quantity ethanol with the objective of obtaining vitivinicultural products with a reduced or low alcohol content and improve its taste balance, respectively.

The degree of alcohol reduction is regulated, the ethanol content may be reduced by a maximum of 20%, assuring that the final content is within the legal range according to the wine definition (see above). The wine subjected to treatment must not have any organoleptic defects nor should the treatment be done in conjunction with any modification in the sugar content in the corresponding musts. Importantly, the techniques allowed (partial vacuum evaporation, membrane and distillation methods) shall be carried out under the supervision of an oenologist or specialised technician to assume responsibility for the whole process.

The new definitions for dealcoholised beverages (OIV-ECO 432-2012) now include:

- *beverages obtained by dealcoholisation of wine*: as a dealcoholised beverage obtained exclusively from wine and with an alcoholic strength by volume below 0.5% v/v. The use of the denomination "dealcoholised wine" has, however, to be allowed by each state member.

- *beverages obtained by partial dealcoholisation of wine*: as a beverage obtained exclusively from wine that has undergone a dealcoholisation treatment and with an alcoholic content equal or above 0.5% and less than the minimum content applicable for that same type of wine. The use of the denomination "partially dealcoholised wine" has, however, to be allowed by each state member.

2.4 Consumer demand for alcohol-reduced wine

Wine consumer demands change as a function of lifestyle fashions, public health policies, social and cultural conventions and actual shifts in taste. This also applies to the amount of alcohol that consumers expect to find in a certain style of wine. A fundamental oenological issue is to know if a certain wine quality and style can be maintained when adapting it to various degrees of alcohol.

OIV's statistical report on the world wine market from 2013 shows that the wine consumption in countries that traditionally produce and wine drinking has reduced, whereas it has been rapidly increasing in new wine markets like Asia - with a 67% increment relative to the year 2000. Moreover the market assists to an increasing demand on healthier options, namely wine with lower alcohol content.

Simultaneously, the same market is in desire of wines aromatically rich and complex, which require grapes phenolic mature, and consequently with higher levels of sugar and thus higher alcohol content in the final product. Reducing the alcohol in the wine does not come without effect in aroma and flavour. On the one hand, the sensation of bitterness, burning and full-mouth is reduced, as well as the perception of acidity and astringency, as the polyphenolic component is affected. On the other hand one could think that the volatile components of the wine would be intensified as the higher the alcohol the higher their volatility, but it is not necessarily so as during the dealcoholisation process part of the volatile compounds are loss (Gómez-Plaza et al. 1999, Pickering 2000, Diban et al 2008). The degree of change in the sensorial characteristics of the partially or dealcoholised wines depends on the amount of alcohol removed and on the technology used.

Despite all the negative effects of alcohol consumption, specially if abusive, moderate consumption of alcoholic beverages, namely red wine has been shown to have, though remaining controversial, beneficial effects to health. Many of the wine components present in red wine, like the potassium and sodium ions are important to the ionic equilibrium of the body, the minerals and acids can help digestion processes and in addition several studies have focused on the high content of antioxidant compounds (e.g., resveratrol) and their protective characteristics due to the synergistic effect of ethanol and the polyphonic compounds (Di Castelnuovo et al. 2002; Chiva-Blanch et al. 2013).

Moreover, some studies have been published about the remaining health benefits of (partially) dealcoholised wine. Lamont et al. (2012) showed that lower ethanol content in wine does not affect its antioxidant properties, by comparing the effect in mice of consuming wine with 12% (v/v) and 6% (v/v) ethanol. Another study concluded that the daily consumption of dealcoholised wine could even improve health by decreasing systolic and diastolic blood pressures and thus preventing hypertension (Chiva-Blanch et al. 2013)

Undoubtedly health issues are on top of the list for low ethanol wine demand. But social issues are as important. Moreover, in many countries the additional ethanol tax can have a strong effect on the drink final price to the consumer but also increases production associated costs, representing an obstacle to international and national markets competitiveness.

Today's consumers have a wide range of possibilities to choose from in what accounts for low ethanol content drinks. Producers have "bombed" the market with easy-to-drink beverages, "wines" included.

Worldwide, and in particular in the so called new world, many are the examples of partially or dealcoholised wine present in the market. In the old world Germany may be the exception, as they have been producing dealcoholised wine for more than a hundred years (e.g. Carl Jung in Rheingau region mentioned above). In Italy, Asti sparkling wine is famous not only for its sweetness (with approximately 70-120 g/L residual sugar) but also for the low ethanol content, usually around 5.5%. The vinification of Asti sparkling wines is based in interrupting the fermentation before it reaches dryness to keep high sugar and low ethanol contents.

In California, dealcoholised wines are relatively common, connected to cosmopolitan healthy trends for consuming wine with less calories and thus reduced alcohol content. Opinions are divergent, but regular wine consumers tend to be prejudicial towards this type of wines. Some of these wines however have received awards in blind tastings (Fig. 5)

From an online research and various opinions from wine related professionals, students, and consumers, many varied products came up. "Wines" from all over the world are now available and reunite different opinions. Sweet wines, sparkling, red, white and rosé, a wide range seems to available. The majority of low alcohol wines being mentioned are however between 9-11% with very few exceptions under these values. Not only because according to the OIV they cannot be called wines but also because critics and consumers and sceptical about their quality.

Many journals, magazines, blogs etc published in the last decade several articles about low-alcohol wines (see appendix for a more exhaustive list of products and articles found). Here below is a selection of some of those wines.

2.4.1 USA

Ariel non-alcoholic wines are sold as a healthy choice. In 1986, according to the company's website (http://www.arielvineyards.com) Ariel blanc was awarded the gold medal at the Los Angeles County Fair, against wines with alcohol (Fig. 5).

FIGURE 5. CALIFORNIA DEALCOHOLISED WINES WITH AWARDS

The wines fermented and made as traditional wines are dealcoholised through a cold filtration process using reverse osmosis. Moreover, Ariel is described to be an alternative to soft drinks and fruit juices, tending to be richer in sugar, sodium and artificial sweeteners, and also with more calories. Also, they attest that "taste and complexity of wine" remains in the final product, as well as all the beneficial anti-oxidant characteristics usually attributed to wines.

Critics and consumers seem to think otherwise. Reed Tucker from the NY Post, published in Jan. 10th 2015 (http://nypost.com/2015/01/10/non-alcoholic-beer-and-wine-are-hot-but-do-they-taste-good/) an article about non alcoholic beverages where he describes Ariel wines as tasting like juice without any of wine's complex flavour or mouth-feel. One other article from Steve Tobak (2007) reported the gold medal winner Ariel blanc as tasteless and undrinkable.

FIGURE 6. WOMEN'S WINE: SKINNYGIRL AND THE SKINNY VINE

Low calorie wines for "cool healthy modern age girls"! Both brands - Skinny girl and Skinny Vine are introduced as low calories wines (Fig. 6), to be consumed at a night with girl friends. The colours, the names, the wording, every detail in the market is to target "pinky girly girls".

The skinny girl wine collection does not even mention the alcohol content, rather expressing the information in terms of calories per serving, adding that it "gives yourself permission to enjoy" a pleasing wine from pinot noir to cabernet sauvignon to chardonnay to pinot grigio, etc. Some of the wines were awarded in the Los Angeles International Wine & Spirits and Women's Wine Competition. The skinny vine collection, under the responsibility of the female assistant winemaker Dawn Wells, are as well introduced with a nutritional fact sheet but also with the respective alcohol content ranging from 7.3% (Mini Moscato) to 8.5% (Slim Chardonnay).

2.4.2 Australia and New Zealand

From Australia and New Zealand that are many examples of low alcohol wines with less than 8% v/v. Mostly whites (or rosés) blends or varietals, there are much less red available options.

Miranda Wines have a reduced and dealcoholised collection (Fig. 7), in which the wines are obtained using spinning cone column technology.

FIGURE 7. MIRANDA SUMMER LIGHT SHIRAZ

Summer Light Shiraz low calorie low alcohol wine as it is introduced in the market according to some reviews is drinkable (Miggin 2013).

Produced in South Australia, the Banrock Station Light blend with only 5.5 % v/v, makes part of a light collection of wines, that according to the wine critics vary in quality.

FIGURE 8. BANROCK STATION LIGHT 5.5%,

2.4.3 Italy

Described as a "delicious, fresh and aromatic dessert wine" this slightly sparkling from Piemonte from Moscato Bianco is only 5% v/v. It is present as a kind of alternative healthy wine also suitable for vegetarians (Fig. 9).

FIGURE 9. ITALIAN ALASIA MOSCATO D'ASTI

2.4.4 Spain

Established in 2001, this designation of origin in the Alava province, Basque region in Spain, is known to produce lower alcohol wines with a minimum of 9.5% v/v (Fig. 10). Generally white, can also be red and rosé. The wines are bottled with their lees, allowing for a second fermentation giving rise to a lightly sparkling wine.

FIGURE 10. DO ARABAKO TXAKOLINA

Produced in Catalonia, Spain by Miguel Torres, Natureo dealcoholised muscat wine came out in an article in the Decanter magazine (2008), as the first dealcoholised wine produced in Spain (Fig. 11). Costs around 7 EUR. Once again critics vary from terrible to amazing.

FIGURE 11. TORRES NATUREO DEALCOHOLISED WINE

DO Jumilla (Spain) reduced alcohol “wine” is a blend of Monastrell, Tempranillo and Petit Verdod with only 6.5% (v/v) (Fig. 12), that ages in french and american oak for 6 months. Its production results from a joint collaboration between the company Bodegas de la Casa de la Ermita and the Universities of Murcia and Cartagena and the CDTI (Centro de Investigación y Desarrollo Tecnológico), in which the grapes were followed from the vineyard to the cellar and harvested with a lower sugar content and thus a reduced potential alcohol content. In the cellar they used low yield yeasts and subjected the must to temperature variations of up to 15ºC in less than 3 hours. The end of the fermentation is followed by a physical process of alcohol reduction. The tasting critics are very disappointing for such a project, referring that the "wine" lacks body and structure, and despite the pleasant aromas in the nose, all is lost in the mouth.

FIGURE 12. 'ALTOS DE LA ERMITA' REDUCED ALCOHOL CONTENT "WINE"

The company's website does not present any information about the wine, however. All it could be found was from general websites (rioja2.com, aprendeacatarvino.wordpress.com and eladerezo.hola.com).

2.4.5 Germany

Low alcohol "wines" are not new in Germany. Many wineries have been producing dealcoholised sparkling for a long time, like Carl Jung (see above). Also many Rieslings, influenced by the cool climate of most of the wine regions in the country have lower alcohol contents than a so called common white.

”B” wines are produced by one of the biggest-selling wine export wineries - Reh Kendermann, in Bingen, Rheinhessen region (Fig. 13). With only 5.5% v/v and around 30% fewer calories (as announced in the website http://www.black-tower.de/en/b-black-tower/b-black-tower-en), is mainly for the external market in UK. As the north american brands, the target is the female consumers from a modern era and with a health-conscious approach to life (http://www.glengarrywines.co.nz).

FIGURE 13. “ B” BY BLACK TOWER WITH 5.5% V/V

Another example that tends to receive better reviews than the B wines is a low alcohol riesling from the Mittel Mosel region, Willi Haag (Fig. 14). One of the reasons behind maybe be the higher content in residual sugar characteristic from this region and wines that counterbalance the low ethanol content and the high acidity.

FIGURE 14. WILLI HAAG 2002 BRAUNEBERGER JUFFER RIESLING KABINETT (8.5 PER CENT),

2.4.6 Portugal

In Portugal as well there are a couple of low-alcohol "wine" products being commercialised. Traditionally, "vinho verde" from the northeast region of Portugal has a lower alcohol content commonly only reaching 9-10% v/v. But market demands are being used by winemakers to try new styles. Nieport and Lancers have done it. Lancers produced the first dealcoholised wine, Free rosé (Fig. 15). Already present in the market as a summer rosé, the alcohol free version is a blend of Portuguese and international varieties (appendix xx) relatively sweet (40g/l) as many of these wines.

FIGURE 15. FREE ROSÉ, LANCERS

Nieport is also investing in low-alcohol wines. As described in their website inspired in Mosel rieslings they produce a sweet 8% v/v Riesling and Muscat (Fig. 16). In both cases, the residual sugar is relatively high, and the fermentation is arrested before it comes to the end.

FIGURE 16. NIEPORT LOW ALCOHOL PROJECTS

There are more examples of low-alcohol wines, but to my knowledge, none is directly comparable in quality and taste to a “normal” wine.

Recently, Lattey et al. (2010) showed that winemaker’s quality on commercial red wines is strongly related to alcohol content, this is, the higher the alcohol and astringency the higher the scores. Bindon et al. (2014) corroborate this preference with a clear choice from the panelist for more alcoholic wines (more than 13% v/v) in detriment of the lower ones (from 12 to 13% v/v). One other study by Bogianchini et al. (2011) analysed commercial low-alcohol wines for their antioxidant activity and phenolic composition impacted by reverse osmosis techniques. Tasters clearly disliked the lower alcohol ones.

Despite the growing interest in the lower alcohol wines there are not, to my knowledge, many studies about wine professionals and wine consumers’ preferences. The available studies report in general a negative opinion about these kind of wines (Meillon et al. 2009, 2010, Schmidtke et al. 2012). A complete and thorough study published in 2014 by Bruwer et al. about low alcohol wine in the UK market, one of the largest markets in the world value wise, showed that the majority of purchase decisions for lower alcohol wines is not the desire to reduce alcohol intake but rather to reduce costs as taxes for ethanol rate have a strong impact in the final price. Lower alcohol wines are expected to be cheaper.

Wine is generally assumed as a complex beverage being more difficult to be accepted with changes such as lower alcohol content, when compared with other less “elite” and “traditional” beverages like beer, cider and RTDs. Also as mentioned above wine quality is often related to alcohol content, which makes low alcohol wines to be looked at as not so good products from the very beginning.

The challenge to make a reduced alcohol wine that looks, smells and tastes as good as a 'normal wine' remains. Aromas, mainly, are lost in the dealcoholising process, specially with some evaporation techniques, thermal techniques are said to impart undesirable 'cooked' and other flavours, reverse osmosis based methods have been reported to be responsible for a loss of flavour intensity and typical 'wine characters', and ethanol itself with taste properties - bitterness and sweetness - in lower proportions in the wine brings, often to a point of imbalance, increasing acidity, bitterness and astringency, and a reduction in 'body' perception. Blending with full-strength wine, pomace, juice or juice concentrate, has shown to improve quality and help regaining aroma and flavour balance in finished wines with reduced alcohol levels. Moreover, phenolic, CO_2 and sugar-free content must be taken into account when optimising the blend.

Research on this topic, of alcohol reduction in wines, will bring to knowledge new and appropriate technologies and techniques to be used in the wine industry allowing the production of partially and/or dealcoholised wine without giving up on phenolic maturation and quality of the wine, thus maintaining the organoleptic properties of the final product.

Chapter 3. Material and Methods

3.1 Wines

Different wines were used for distinct parts of the research, this is, the wines used in the tastings were different from the ones used during the dealcoholisation trials.

3.1.1 Wines for the tastings

The wines used in this study were vinified and donated by a local winery named Rotkäppchen in Eltville, Germany. Three wines - cv. Riesling, cv. Dornfelder and Mediterranean Cuvée (MD Cuvée, unknown selection of varieties) - were selected, after tasting, from an available range of dealcoholised and their equivalent untreated wines. For each type of wine, coming from different original batches, we had one set with "normal" alcohol content and one other dealcoholised to approximately 0.5% using industrial scale vacuum rectification (wine treatment was carried out in the company's facilities in Rüdesheim, Germany).

3.1.2 Wines for the dealcoholisation trials

Two wines were used in this part of the research. A red wine of cv. Spätburgunder (Pinot Noir) and a white blend from current Italian varieties were used to carry on lab scale alcohol reduction using membrane technology Osmotic Distillation (see detailed process below).

3.2 Physicochemical Analyses

For each base wine physicochemical analyses were made. The analyses followed OIV protocols and were done in duplicate when necessary, being the results presented in such cases the arithmetical average of the two obtained values (marked with an *).

A total of 20 parameters were analysed (see list below), chosen for their relevance as wine quality descriptors in this project context.

Physicochemical parameters measured:

Actual Alcohol (g/L and % vol)

Total Alcohol (g/L and % vol)
Total Extract (g/L)
Sugar Free Extract (g/L)
Residual Extract (g/L)
Sugar Before Inversion (g/L)
Residual Sugar (g/L)
pH
Total Acidity (g/L)
Volatile Acidity (g/L)
Free SO_2 (mg/L)
Total SO_2 (mg/L)
Density (20/20)
Refraction Number
Tartaric Acid (g/L)
Total Phenols (mg/L)
Reductone (mg/L)
Colour (Wavelenght absorbance)

All analyses were done in the oenological laboratory in Geisenheim University under the surveillance of the responsible laboratory technician. Safety and security rules were followed and previous to the first day of lab work, I was required to read and sign a list of safety and emergency rules to be followed in all times (namely, because I was pregnant during the period of this research work some analysis had to be done by someone else to avoid contact with possible contaminant and dangerous products).

For personal safety reasons - pregnancy, due to some chemical reagents required in the protocol, reductone analysis was done by someone else. Measuring reductones (sometimes also called reductants), especially in red wines, is of extreme importance to accurately determine the real free SO_2 content (Jančářová et al. 2014). Reductones are mainly represented by ascorbic acid being their content expressed as mg/L of ascorbic acid. When measuring free SO_2, present reductones are also detected and can mistakenly lead to a higher free SO_2 reading and, consequently, an insufficient sulphuration of wines or musts. When evaluating SO_2 all values were corrected for reductone content.

Colour of the wines was measured with a spectrophotometer by measuring the samples absorbance between 380 to 770 nm wavelength. Chromatic characteristics of a wine are defined by the chromaticity coordinates: clarity (L*), red/green colour component (a*), and blue/yellow colour component (b*). In other words, this CIELab colour or space system is based on a sequential or continuous Cartesian representation of 3 orthogonal axes: L*, a* and b*. Coordinate L* represents clarity (L* = 0 black and L* = 100 colourless), a* green/red colour component (a*>0 red, a*<0 green) and b* blue/yellow colour component (b*>0 yellow, b*<0 blue) (OIV 2006 and Bain 2009; Fig. 17). Moreover, some chromatographic parameters were calculated. Intensity of the colour is given by the some of absorbances of 420, 520 and 620 nm wavelengths - I = A420 + A520 + A620. Nuance (or colour hue) is conventionally given by the ration A420/A520.

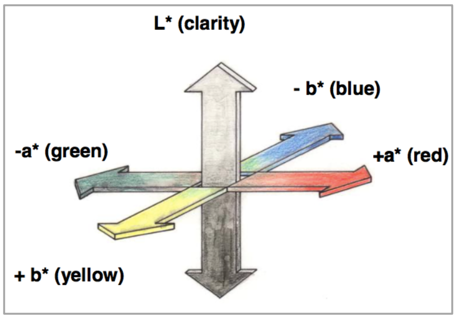


FIGURE 17. DIAGRAM OF COLOURIMETRIC COORDINATES ACCORDING TO COMMISSION INTERNATIONALE DE L'ECLAIRAGE (CIE, 1976 FROM OIV 1/2006)

3.3 Dealcoholisation of the wines by Osmostic Distillation

The experimental set up of the osmotic distillation (OD) system used during this project is shown in Fig. 18. The OD machine is connected to the wine (feed, left side in a glass jar) and water (strip, bottom right in a 250L plastic recipient) recipients.

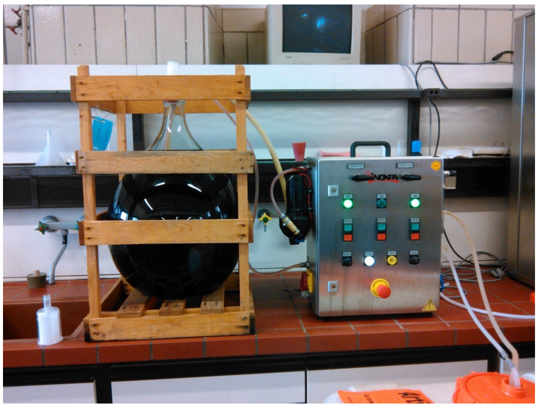


FIGURE 18. OSMOTIC DISTILLATION SET UP (PHOTO BY MOREIRA 2015).

We used the WineBrane LAB GAS/ALC model from INOXPA® (Banyoles/Spain) with a membrane contactor from Liqui-Cel® (see detailed information of the equipment in Appendix 1). The wine (feed) was pumped by a membrane pump with a flow rate capacity of 300L/h and the water (strip) pumped by a peristaltic pump with a flow rate capacity of 120L/h (Schmitt et al. 2014). The trials took place at room temperature and dealcoholisation was carried out until the wine reached an approximate 3.5% (v/v) alcohol content.

Every hour, except for the overnight period, alcohol contents, from both feed and strip, were measured using an ebuliometer (Appendix 1), previously calibrated following manufacturer instructions.

Many factors affect the alcohol removal performance but also the volatile compounds loss from the wine, i.e., volumes ratio of feed/strip, feed and strip velocity, and temperature. In our experiments, feed and strip velocity were constant and temperature variation was minimal and depreciable (cellar temperature varied little during the whole research period). Our main focus was on the effect of feed/strip volume ratio and the temperature of the water (strip), and thus different scenarios were set up as described below (see table IV below).

TABLE IV. VOLUME RATIO OF FEED/STRIP FOR ALCOHOL REMOVAL TRIALS USING OSMOSTIC DISTILLATION

Wine (ratio feed/strip)	Feed (wine volume, L)	Strip (water volume, L)	Observations
Spätburgunder (1:1)	50	50	no data available
Spätburgunder (1:4)	50	200	Cold water
White Italian (1:3)	50	150	warm water

3.4 Microbial spoilage of low alcohol wines

One of the problems low alcohol wines may pose is how to be conserved and avoid microbial spoilage over time. As mentioned before, ethanol as an important role as an anti-microbial agent, and reduced levels may lead to higher risks of contamination. Are the current legal limits for protective/anti-microbial agents, like sulphur among others, enough to maintain light wines protected?

At a preliminary stage we will verify if the so-called "normal" levels of sulphuration are enough to avoid microbial spoilage. Is an approximate concentration of 50 mg/L of free SO_2 enough?

3.4.1 Blending and Bottling

Two alcohol content combinations were used in the microbial spoilage trials representing the extremes we were working with: 4% and 8% (v/v). Blends were done (23 March 2015) using alcoholised and dealcoholised base wines mentioned above. The ethanol content of each wine was measured previously using an ebuliometer. Free SO_2 corrected for reductone presence was measured and blended wines were protected with sulphur dioxide, added in liquid solution, aiming to get approximately 50 mg/L, immediately afterwards. New free and total SO_2 measurements were done 24 hours after blending, and levels were corrected when needed.

Sterile bottling was carried out 3 days later (26 March 2015) using the bottling line from the Geisenheim Oenology Department (Fig. 19). Previous to bottling, all bottles were cleaned and disinfected with a 2% sulphur solution. Wine density was measured before and after to avoid mixed phases that may occur during bottling. Seven bottles of each wine MD cuvée, Riesling, Dornfelder and Spätburgunder 4% and 8% were bottled. The bottles were placed in the underground cellar at controlled humidity and temperature levels.

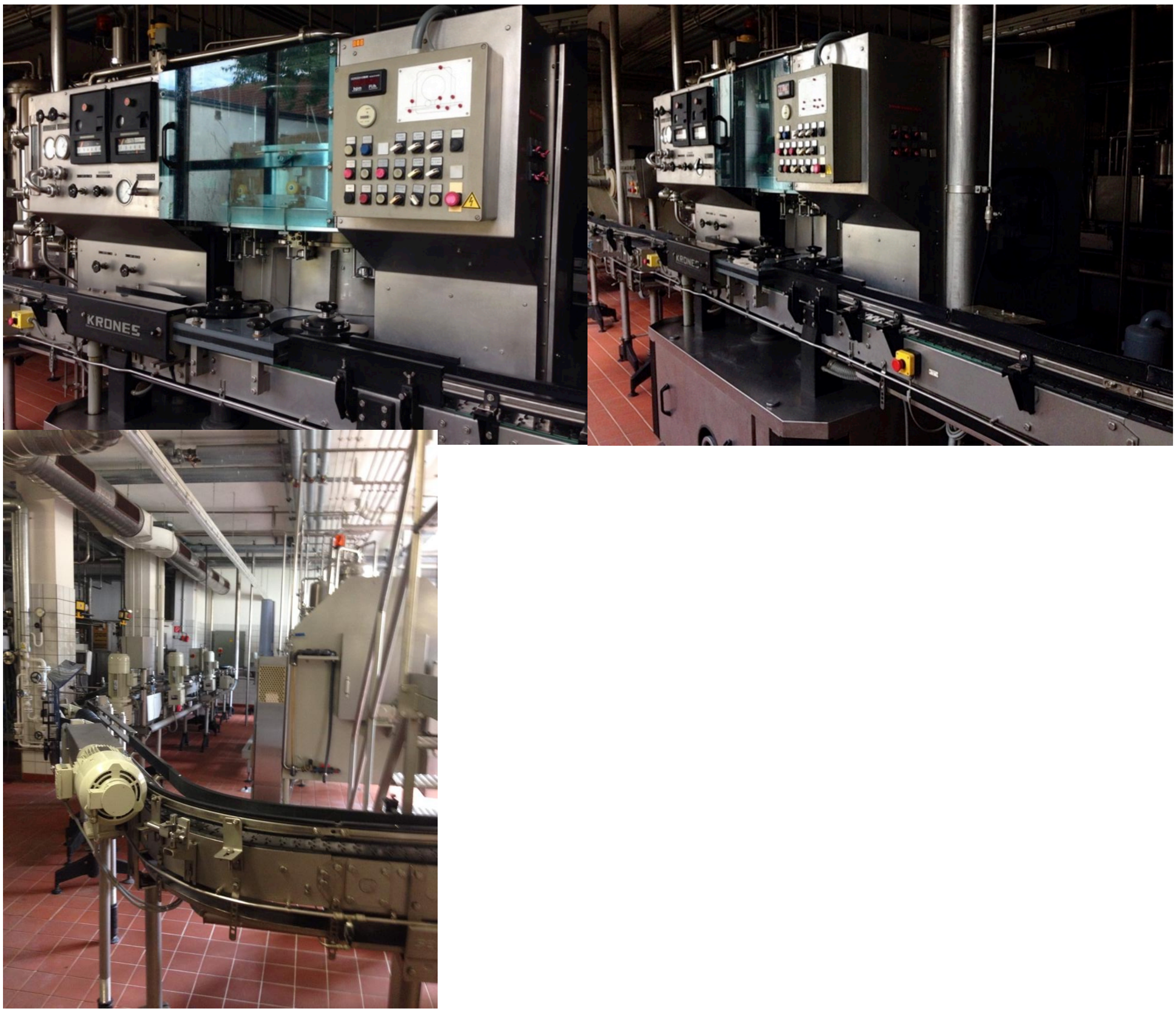


FIGURE 19. BOTTLING LINE FACILITIES AT GEISENHEIM UNIVERSITY

3.4.2 Microbiological tests

Three microbiological tests were made two, five and seven weeks after bottling to test for suitability of sulphuration levels and possible microbiological contamination. To test for sterility of the bottled samples we used the membrane filter method (material from Sartorius ®), in which the content of one bottle (approximately 0.8 L) of each wine was sterile filtered under vacuum conditions, using a membrane filter from Whatman with pore size of 0.6µm (Fig. 20 left). The membrane filter was then placed inside a petri dish with a wetted sterile nutrient pad (Fig. 20 right), and placed in an incubator for three days at 25ºC, following manufacturers instructions. The nutrient pad - Wort-NPS - is recommended for yeasts and molds detection (Fig. 20 centre).

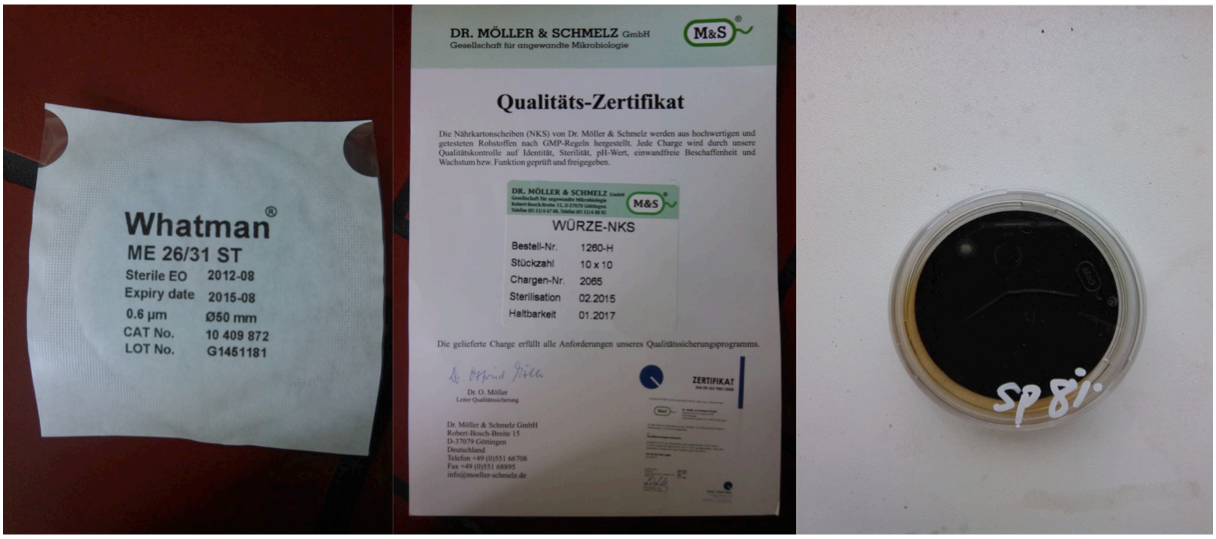


FIGURE 20. FILTER PACKAGE (LEFT), NUTRIENT PAD (CENTRE) AND PETRI DISH WITH FILTER AND NUTRIENT PAD (RIGHT) AFTER INCUBATION PERIOD (NOTE ONE COLONY ON THE TOP LEFT OF THE FILTER)

The petri dishes were verified 3 days later. Photographies were taken from all of them, and the media were discarded. As described by the manufacturer, yeasts are expected to develop smooth white or coloured colonies and molds velvety or fluffy cotton-like colonies.

3.5 Tasting/Sensorial analysis

The objective here is multifold: 1) to determine if there is a sensorial difference between partially dealcoholised wines with different ethanol contents, 2) evaluate sensorial preferences in partially dealcoholised wines, and 3) test what is the ethanol threshold perception among trained tasters.

These tastings are of extreme importance to understand consumer judgement about partially dealcoholised wines. Are consumers convinced about their quality when compared to an untreated wine? Based on sensorial descriptions what attributes can or should be altered when producing a reduced alcohol wine? To better understand consumer opinion about reduced alcohol wines, tastings were organised with experienced tasters from Geisenheim University (Germany).

All sensory analyses took place at a dedicated standardised tasting room, with controlled temperature (~20ºC), and individual slots at Geisenheim University Campus. The panel had access to fresh water and plain crackers to consume in between tastings to clean the palate and avoid sensorial fatigue. All answers were to be written down in a computer-produced sheet that randomised all the tastings and sample ID codes (Appendix 3).

3.5.1 First Tasting

To test the hypothesis that humans, even trained panelists, cannot sensorially discriminate 2% or less in the alcohol content of wines. Recent studies report that only above 2% difference in alcohol content rapports a sensorial perception (Schmitt et al 2013), even though previously, Yu and Pickering (2008) had reported for Chardonnay and Zinfandel wines a ethanol threshold ranging from 0.5% to 1.31% with a trained panel.

The tasting took place on the April 23rd, 2015. Three different wines were used - two whites Riesling, MD Cuvée and one red, Dornfelder - with alcohol levels adjusted to 4%, 6% and 8% (v/v) by blending the untreated wines with the dealcoholised ones (see details above and Table V). The blends were prepared the day before of the tasting, bottled immediately after, and kept in the cellar at an average temperature of 15ºC.

TABLE V. BASE WINES USED FOR SAMPLE PREPARATION

Alcohol content	Riesling	MD Cuvée	Dornfelder
Normal wine	11.1%	10.7%	11.6%
Dealcoholised wine	0.4%	0.3%	0.4%

Note: alcohol content was measured by refractometric and density methods.

A discrimination method - triangular test - was applied to determine sensorial differences between two types of wine, a reduced alcohol wine and an untreated wine. This kind of tests are widely applied in such cases and have proved to be efficient and provide clear results (e.g. Diban et al. 2008, Aguera et al. 2010). The first objective was to determine wether panelists were able to recognise the wine that was different in the triad. The second objective was to identify which wine was preferred by the panelists who correctly identified the different wines.

Each taster was given three samples simultaneously, in glasses, and asked to identify the different sample in terms of aroma and flavour, and afterwards choose their favourite.

There were 3 different samples with different alcohol contents. All possible combinations were tested: 4% against 6%, 4% against 8%, and 6% against 8%, a total of 9 triangular tests. The samples and tests were computer randomised to avoid human bias.

The second part of the tasting was an In-Out test (pass-fail) to evaluate an overall quality rating by the taster. The tasters were asked to give their opinion about single samples. Previous to each test, a control wine was presented and briefly described by Matthias Schmitt. Following this step, three samples (with different alcohol contents) were presented to the panelists to be evaluated in comparison to the control wine. The objective was to, in comparison to the control wine, evaluate the expectation of the taster about a wine of a specific type. If the wine was as expected than the panelists should choose ‘well in’, if on the contrary they should choose ‘well out’. In case of an intermediate opinion, the panelists could choose either ‘just in’ or ‘just out’, accordingly (appendix xx).

3.5.2 Second tasting

The second tasting, based on the results from the 1st tasting, had the objective to obtain a sensorial description of a “normal” (control) wine non-dealcoholised and a partially dealcoholised wine.

The panellists were presented two wines at each time (Riesling, MD Cuvée and Dornfelder), a control wine (alcohol content between 10-11%) and a partially dealcoholised wine with 4% (v/v). Previous to each tasting, the panel was given the control wine to taste and a short description of the type of wine in terms of grape varieties and sensorial attributes to be evaluated during the tasting.

The panellists were asked to evaluate a randomly chosen wine in terms of 5 attributes: fruitiness, body/full sensation, sourness, sweetness and bitterness by using a numerical scale ranging from -5 to 5, in which ‘0’ (zero) stand for optimal, positive values better than expected (balanced) and negative values worse than expected (unbalanced) (appendix xx).

Chapter 4. Results

The main goal of this research work was to evaluate preliminary factors for light wine acceptance and production. Alcohol reduced wines produced by vacuum rectification (industrial scale) and osmotic distillation (experimental lab scale) were used. Osmotic distillation trials were run to test for the efficiency of the technology. Sensorial tastings using commercial wine were carried out to evaluate panelists opinion and ethanol perception thresholds. All experiments and tastings were done in the University of Geisenheim (Germany) in collaboration with ISA, University of Lisbon (Portugal).

4.1 Dealcoholisation of the wines by Osmotic Distillation

Two trials of wine dealcoholisation were done by using osmotic distillation technology. In the first trial we used was a cv. Spätburgunder wine with a initial alcohol content of 11.9 g/L (v/v) (measured with an ebuliometer). The dealcoholisation was carried out until obtaining an ethanol content close to 4%, which took approximately 44 hours (Fig. 21). As it can be seen in the graph the gain in ethanol of the strip does not follow the loss in the feed wine, due to a partial evaporation and loss of the volatile compounds. As time passed it could be noticed, by direct smelling, an increase of the aromas in the strip water (no analysis was done in this study to the aroma compounds present in both feed and strip).

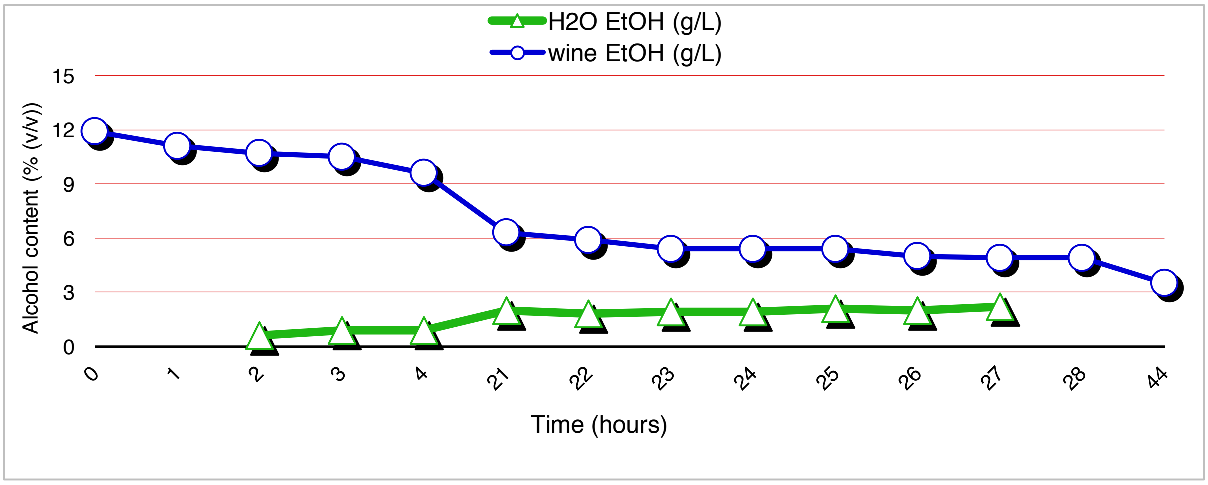


FIGURE 21. ETHANOL CONTENT IN BOTH FEED (WINE) AND STRIP (WATER) DURING DEALCOHOLISATION BY OSMOTIC DISTILLATION - 1ST TRIAL MARCH 17, 2015. INITIAL ETHANOL CONTENT OF WINE - SPÄTBURGUNDER: 11.9 G/L. RATIO

FEED/STRIP - 1:4. X-AXIS REPRESENTS THE TRIAL TIME FROM MOMENT '0' TO THE FINAL ETHANOL CONTENT MEASUREMENT.

The second dealcoholisation treatment was done to a white wine with an initial alcohol content of 10.1% (v/v) (measured with an ebuliometer) (Fig. 22). The osmotic distillation was interrupted when the wine reached 3.8% (v/v), around 40 hours after the trial started - showing a slightly faster rate of dealcoholisation than the 1st trial. In this case, the ratio feed/strip was 1:3 and the strip water was warm (initial temperature of 34ºC). Both wine and water ended up with identical temperatures (final temperatures were 24.4ºC and 24.2ºC, respectively.

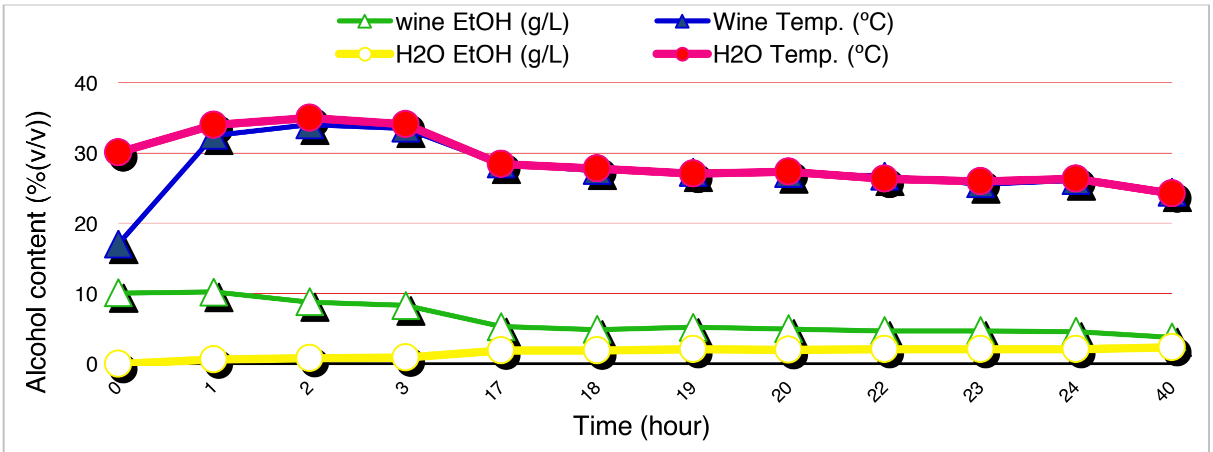


FIGURE 22. ETHANOL CONTENT IN BOTH FEED (WINE) AND STRIP (WATER) DURING DEALCOHOLISATION BY OSMOTIC DISTILLATION - 1ST TRIAL MARCH 17, 2015. INITIAL ETHANOL CONTENT OF WINE - WHITE CUVÉE: 10.1 G/L. RATIO FEED/STRIP - 1:3. STRIP WATER INITIAL TEMPERATURE: 30.1ºC. X-AXIS REPRESENTS THE TRIAL TIME FROM MOMENT '0' TO THE FINAL ETHANOL CONTENT MEASUREMENT.

TABLE VI. PHYSICO-CHEMICAL PARAMETERS OF TWO WINES: ONE UNTREATED (WITH ETOH) AND ANOTHER PARTIALLY DEALCOHOLISED (REDUCED ETOH) BY OSMOTIC DISTILLATION.

Parameter	reduced EtOH	With EtOH
Actual alcohol (g/L)	3,8	10,1
Sugar before inversion (g/L)	0,7	1,5
pH	3,1	3,0
Total acidity (g/L)	7,0	8,1
Volatile acidity (g/L)	0,216	0,264
Free SO2 (mg/L)	14,6	42,5
Total SO2 (mg/L)	85,6	141,6
Density (20/20)	0,9945	1,0092

Parameter	reduced EtOH	With EtOH
Conductivity (µS/cm)	1544	2725
Saturation Temperature (ºC)	62	58

The base wine used in the second trial and the resulting partially dealcoholised wine were subjected to physico-chemical analyses (Table VI). Volatile acidity, as expected, decreased in the dealcoholised wine as well as density and sugar content. Total acidity also decreased with a slight increment of the pH in the dealcoholised wine. In terms of tartaric stability neither of the wines were stable, with conductivity values higher than 60 µS (Schmidt 2009).

4.2 Physicochemical analysis

The six wines to be used in the sensorial tastings, untreated and dealcoholised, were analysed for 19 parameters as soon they arrived to the university (Table VII). Two measurements were done for some of the measurements (sugar before inversion, pH, total acidity, volatile acidity, free SO_2, total SO_2, and total phenols; indicated in the table with an *) being the results showed an average of the two values obtained.

TABLE VII. PHYSICOCHEMICAL ANALYSIS TO THE 6 WINES USED IN THE SENSORIAL TASTINGS.

	MD Cuvée		Riesling		Dornfelder	
Parameter	**w/ EtOH**	**No EtOH**	**w/ EtOH**	**No EtOH**	**w/ EtOH**	**No EtOH**
Actual Alcohol (g/L)	84,40	7,40	87,80	8,00	91,50	9,90
Actual Alcohol (% vol)	10,70	0,90	11,10	1,00	11,60	1,20
Total Alcohol (g/L)	85,00	7,90	87,90	8,40	93,00	11,10
Total Alcohol (% vol)	10,80	1,00	11,10	1,10	11,80	1,40
Total Extract (g/L)	21,90	34,30	21,40	37,10	26,10	39,40
Sugar Free Extract (g/L)	20,70	33,40	21,20	36,20	22,90	36,70
Residual Extract (g/L)	6,80	21,70	6,30	21,60	10,40	25,90

	MD Cuvée		Riesling		Dornfelder	
Parameter	**w/ EtOH**	**No EtOH**	**w/ EtOH**	**No EtOH**	**w/ EtOH**	**No EtOH**
Sugar Before Inversion (g/L) *	2,20	1,85	1,15	1,90	4,30	3,70
Residual Sugar (g/L)	1,20	0,90	0,20	0,90	3,20	2,70
pH *	3,10	3,05	3,00	2,70	3,50	3,40
Total Acidity (g/L) *	7,80	8,60	8,65	11,60	5,75	7,10
Volatile Acidity (g/L) *	0,34	0,41	1,51	0,9360	0,62	0,85
Free SO_2 (mg/L) *	7,50	8,50	17,50	21,35	45,50	134,00
Total SO_2 (mg/L) *	81,50	114,50	74,00	117,70	93,00	271,30
Density (20/20)	0,99	1,01	0,99	1,0115	0,99	1,01
Refraction Number	37,40	24,10	38,00	25,30	40,70	26,60
Tartaric Acid (g/L)	2,76	3,67	3,01	4,21	2,95	2,33
Total Phenols (mg/L) *	225,50	264,50	209,00	330,00	1923,50	2026,50
Reductone (mg/L)	5,00	7,00	8,00	7,00	31,00	28,00
Conductivity (µS/cm)	1561,00	3038,00	1472,00	2909,00	1501,20	3439,00
Saturation Temp. (ºC)	107,00	164,00	51,00	75,00	68,50	188,00

The wines subjected to dealcoholisation by industrial vacuum rectification suffered, an apparent, alteration in some of the measured parameters. Caution should be taken when analysing these values as the pair of wines (untreated and dealcoholised) do not come from the same original batch.

Dealcoholisation caused an increase in total extract, sugar free extract and residual extract in all three cases. Total phenols also showed an increment in the dealcoholised wines.

Residual sugar, as shown on table VII, showed a lower value for riesling untreated wine when compared to the dealcoholised one, but once again no direct comparison can be made as they were not originated from the same base wine.

Spectra from the six wine samples are shown in figures 23, 24 and 25. Table xx shows colour intensity and hue (nuance) calculation results (see methods for details). The white wines - MD Cuvée and Riesling - absorption spectra are similar in profile (Fig 23 and 24) with similar ranges of intensity of absorption for the measured wavelengths. Both MD Cuvée wines (untreated and dealcoholised) show slightly higher colour intensity compared to the Rieslings.

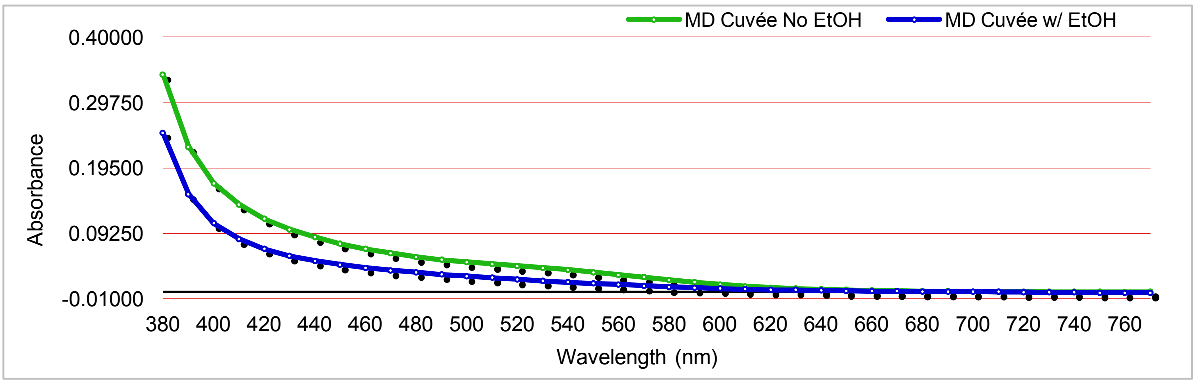


FIGURE 23. ABSORBANCE SPECTRA OF MD CUVÉE WINES, UNTREATED AND DEALCOHOLISED.

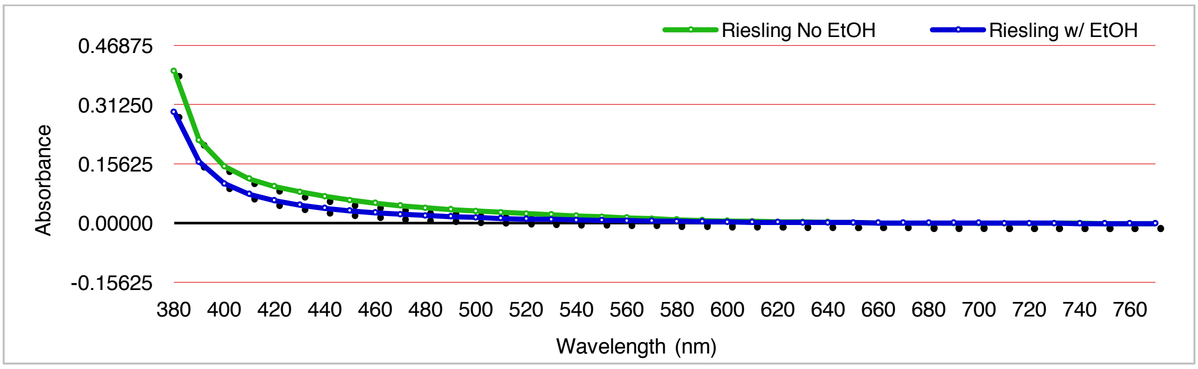


FIGURE 24. ABSORBANCE SPECTRA OF RIESLING WINES, UNTREATED AND DEALCOHOLISED

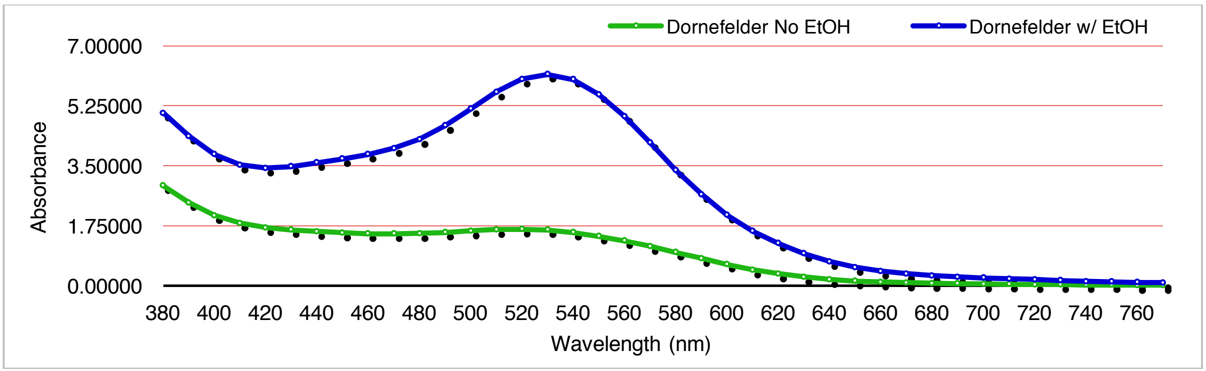


FIGURE 25. ABSORBANCE SPECTRA OF DORNFELDER WINES, UNTREATED AND DEALCOHOLISED.

The red wine (Dornfelder), as expected, shows a different absorption spectrum with higher values of intensities of absorption and an absorbance peak between 460 and 580 nm, much more obvious in the untreated wine (Fig. 25). This peak is naturally explained due to the absorption of anthocyanins present only in red and rosé wines (and absent in whites).

In table VIII is also displayed the measured colour parameters for all six wines. Here again, Dornfelder shows the lowest values for L* (i.e., closer to black than to white); a* value is an indication of the redness or greenness of the colour, the higher the value the redder is the wine - Dornfelder samples have much higher values than the white wines.

TABLE VIII. CIELAB COLOUR, COLOUR DIFFERENCE, COLOUR INTENSITY, AND HUE OF WINE SAMPLES

	MD Cuvée		**Riesling**		**Dornfelder**	
Parameter	**No EtOH**	**w/ EtOH**	**No EtOH**	**w/ EtOH**	**No EtOH**	**w/ EtOH**
X	88,710	91,548	90,813	92,676	19,091	3,250
Y	93,140	96,567	95,904	98,101	11,191	1,391
Z	89,685	96,858	93,103	99,181	2,868	0,021
L*	97,284	98,657	98,394	99,261	39,900	11,899
a*	0,744	-0,011	-0,204	-0,593	52,101	42,156
b*	6,922	4,395	6,472	3,904	36,582	36,558
420 nm	0,115	0,068	0,096	0,059	1,701	3,430
520 nm	0,041	0,020	0,025	0,011	1,653	6,023
620 nm	0,007	0,004	0,003	0,001	0,351	1,240
Intensity	0,164	0,092	0,124	0,071	3,706	10,693
Nuance/Colour Hue	2,773	3,370	3,856	5,601	1,029	0,569

As for negative a* indicate a greener colour, in our case the two Rieslings showed the most negative values. b* is an indication of the yellow-blue colour of the wines, all samples showed positive values indicative of their yellowness.

4.3 Microbial spoilage of low alcohol wines

To evaluate the efficiency of sulphur dioxide in the conservation during storage of reduced alcohol wines, bottled wine was subjected to microbial spoilage tests two, five and seven weeks after bottling.

No contaminations were observed for the white wines - MD Cuvée and Riesling (Figs. 26, 27 and 28). After five weeks Spätburgunder 8% sample (Fig. 27) showed signs of contamination. According to the manufacturer information, such kind of colony, is likely to be a mold contamination. Further analysis needed to be done to ensure its origin.

The third microbial spoilage test, done seven weeks after bottling, also showed some colonies development - most likely molds. Samples from Dornfelder 8% and, again, Spätburgunder 8% (Fig. 28). The third test was done in duplicate, as the second one had revealed contamination signs. The three samples with colony development, by coincidence (or not), two of them were from in petri dishes marked with an *, i.e., all these samples came from bottles from which was removed a 25 ml sample for free SO_2 measurements (table IX).

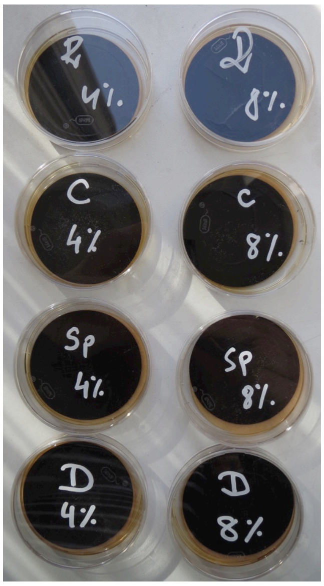


FIGURE 26. MICROBIAL SPOILAGE TRIAL TWO WEEKS AFTER BOTTLING

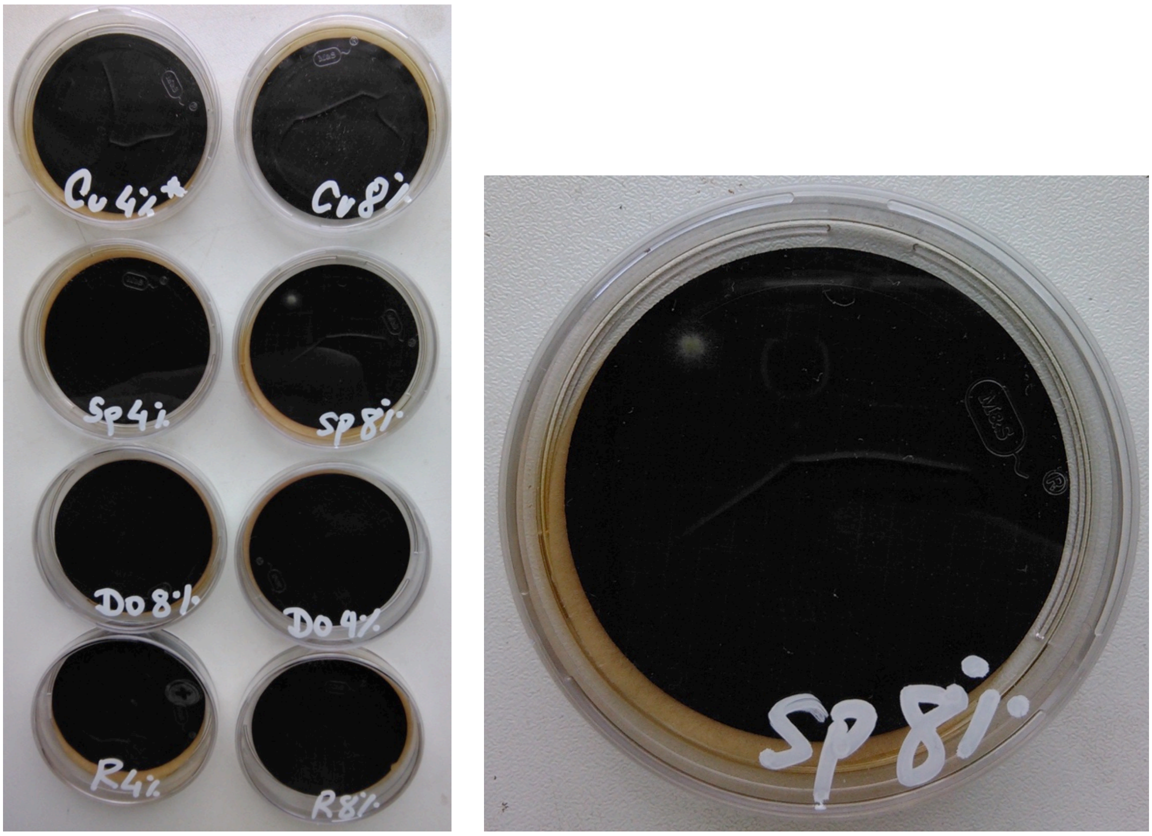
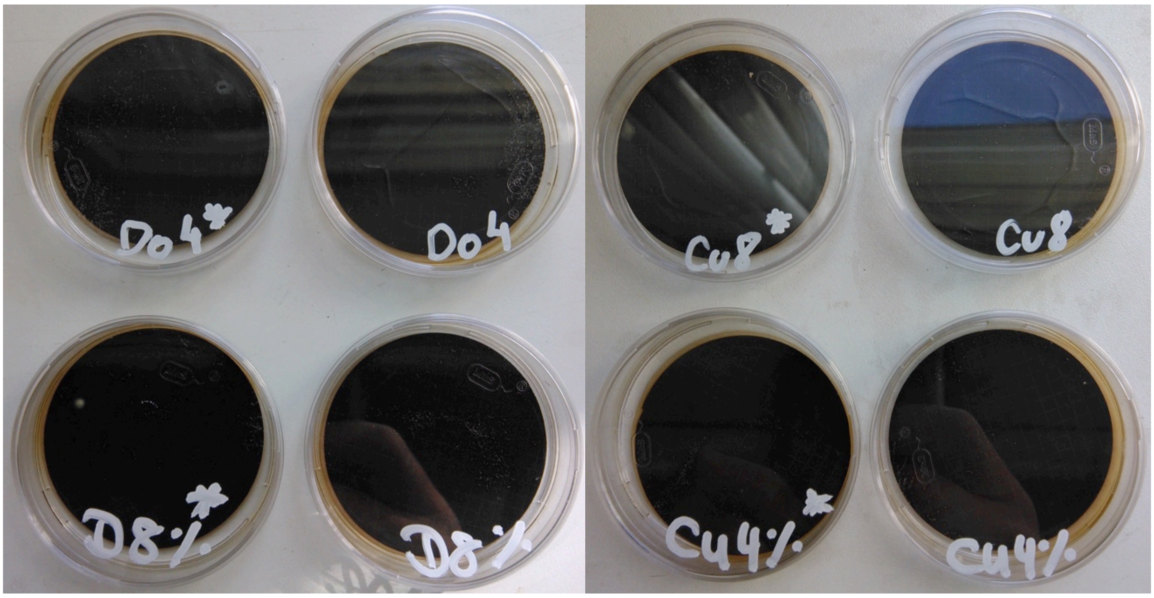


FIGURE 27. MICROBIAL SPOILAGE TRIAL FIVE WEEKS AFTER BOTTLING



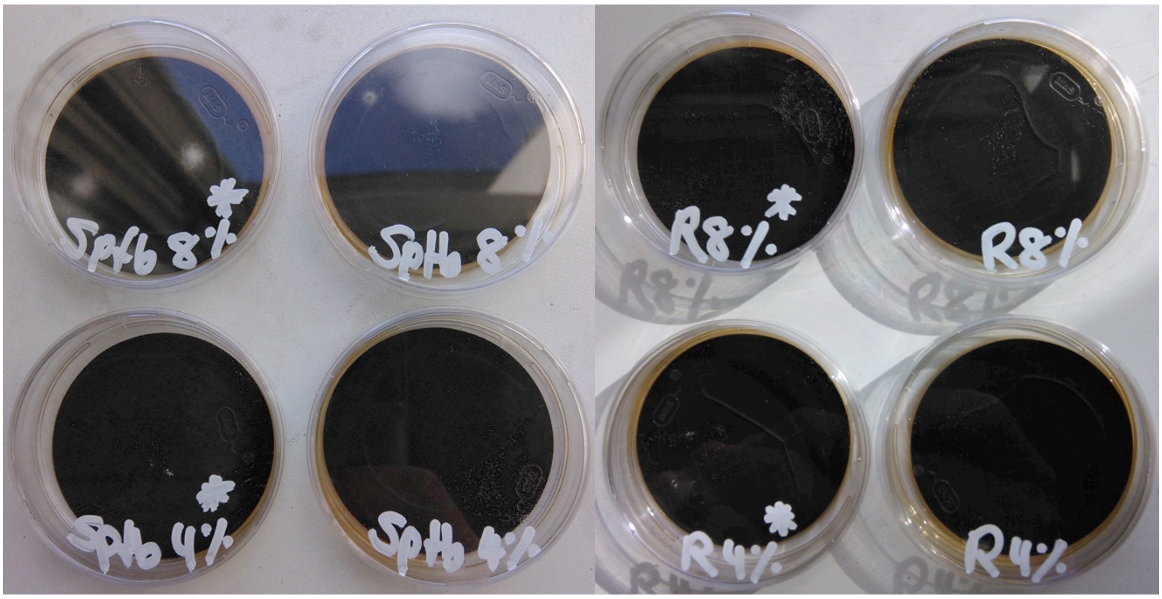


FIGURE 28. MICROBIAL SPOILAGE TRIAL SEVEN WEEKS AFTER BOTTLING

After seven weeks of storage free SO_2 (mg/L) measured in the bottled wine showed some variation, apparently unrelated to alcohol content (table IX). Previous to bottling free SO_2 was measured and corrected for values close to 50 mg/L if under these values (marked with *) (higher concentrations were not corrected if within the legal limits). All samples suffered a reduction in the free SO_2 concentrations, being the most severe reduction observed in the MD cuvée 4%.

TABLE IX. FREE SO_2 (MG/L) MEASUREMENTS PREVIOUS TO BOTTLING AND AFTER SEVEN WEEKS OF STORAGE. * INDICATES SAMPLES CORRECTED FOR FREE SO2 PREVIOUS TO BOTTLING (TO APPROXIMATE CONCENTRATIONS OF 50 MG/L).

<table>
<tr><th></th><th colspan="3">free SO_2 (mg/L)</th></tr>
<tr><th>Wine Sample</th><th>before bottling</th><th>at bottling</th><th>7 weeks after</th></tr>
<tr><td>**MD Cuvée 8%**</td><td>11.9*</td><td rowspan="4">~50</td><td>38.3</td></tr>
<tr><td>**MD Cuvée 4%**</td><td>11.0*</td><td>18.8</td></tr>
<tr><td>**Riesling 8%**</td><td>25.6*</td><td>30.2</td></tr>
<tr><td>**Riesling 4%**</td><td>25.4*</td><td>44.0</td></tr>
<tr><td>**Dornfelder 8%**</td><td>65</td><td>65</td><td>59.8</td></tr>
<tr><td>**Dornfelder 4%**</td><td>87</td><td>87</td><td>83.2</td></tr>
</table>

	free SO_2 (mg/L)		
Wine Sample	**before bottling**	**at bottling**	**7 weeks after**
Spätburgunder 8%	19*	~50	41.3
Spätburgunder 4%	54	54	52.1

4.4 Sensorial Analysis

The aims of the sensorial tastings were to test: 1. ethanol perception thresholds; 2. reduced alcohol wine preference, and 3. sensorial description of reduced alcohol wines. Two tasting sessions took place in which three different types of wine - MD cuvée, Riesling and Dornfelder - with different alcohol contents were tasted.

4.4.1 First Tasting

The first tasting was meant to evaluate the ethanol perception threshold of a trained panel, i.e., the minimum difference in ethanol content that a panel of tasters can discriminate between two different wines. The sensory triangular test carried out to evaluate wether the panel could or not discriminate between two wines of different alcohol content - either 2% or 4% (v/v) difference - was formed by 24 tasters, of which 13 females and 11 males, from 7 different nationalities, with an age range from 19 to 40 (average~26) years, were present (table X).

For each triad combination, the proportion of correct answers as wells as the probability associated of (binomial law, $p=1/3$) are shown in table XI. For statistical reasons only 23 complete tasting sheets were taken into account. For a 23-member panel, the difference between samples was significant only if the number of corrected answers was above 11 ($p<0.05$) - this means that the tasters were able to identify the different sample.

TABLE X. GENDER AND NATIONALITY OF THE FIRST TASTING PANEL.

Nationality	Female	Male
Canada	1	0
France	1	0
Germany	6	6
Holland	0	1
Italy	3	1
Spain	1	1
Switzerland	0	1
Ukraine	0	1
USA	1	0
Total (n=24)	13	11

The triangular tests showed that differences of 2% or less in alcohol content could not be told apart by the tasting panels. In both cases, when asked to discriminate between 4% and 6%, or between 6% and 8%, the number of tasters that answered correctly was not significant. The exception was with the Dornfelder wine where it was statistically significant the number of tasters able to distinguish between the wines with 6% and 8% (table XI). The number of correct answers distinguishing between wines with 4% and 8% was highly significant for both MD cuvée and Dornfelder wines, but not for Riesling (Table XI). The results suggest that only a difference of more than 2% in wines subjected to alcohol reduction by vacuum distillation is perceptible, at least for the 3 varieties/blends tested.

TABLE XI. NUMBER OF CORRECT ANSWERS ON THE TRIANGULAR TESTS OF THE 1ST TASTING (N=23 TASTERS).

	Riesling	MD Cuvée	Dornfelder
4% / 6%	11	11	8
4% / 8%	7	20**	20**
6% / 8%	10	11	12*

Note: for alpha=0.05 * significant and ** highly significant

Interestingly, when analysing the correct answers by gender (female vs male), with only two exceptions (grey fill cells, table XII), females had, consistently, more correct answers than males.

When asked for the preferred wine, within the combinations where the number of correct answers was statistically significant (table XIII), the panelists preferred the wines with lower alcohol content - 4% (v/v). Among the tasters that correctly distinguished between the Dornfelder 6% and 8% (v/v), however the majority (not significant) preferred the wine with higher alcohol content (8% (v/v).

TABLE XII. NUMBER OF CORRECT ANSWERS BY GENDER ON THE TRIANGULAR TESTS OF THE 1ST TASTING (N=23 TASTERS, F=13, M=10).

	Riesling		MD Cuvée		Dornfelder	
	F	M	F	M	F	M
4% / 6%	8	3	7	4	4	4
4% / 8%	5	2	12	8	12	8
6% / 8%	5	5	7	4	8	4

TABLE XIII. PREFERENCES INDICATED BY THE PANEL BETWEEN WINES WITH DIFFERENT ALCOHOL CONTENT DURING THE TRIANGULAR TEST (N=23).

Combination	Riesling	MD Cuvée	Dornfelder
4% / 6%			
4% / 8%		14/6	17/3*
6% / 8%			2/9

Note: for alpha=0.05 * significant and ** highly significant.

The results for the in-out test to evaluate tasters' personal opinion about the typicality of the tested wine are shown if table XIV. The value range from '1' the lowest value meaning a positive opinion (well-in in the tasting sheet, appendix xx) to '4' the highest value - standing for the least appreciated (well-out in the tasting sheet, appendix xx). The results showed that higher alcohol content wines were always preferred (lower values in bold) to lower alcohol ones, contradicting the results from the triangular tests (see above). Between genders there were no significant differences or trends in choice.

TABLE XIV. IN-OUT TEST RESULTS. WINES MORE APPRECIATED SHOW LOWER VALUES (IN BOLD). VALUES PRESENTED ARE MEAN±STANDARD DEVIATION (TASTERS N=23)

Ethanol %	Riesling	MD Cuvée	Dornfelder
4%	2,30±0,69	3,09±0,65	3,22±0,98
6%	2,52±0,88	2,52±1,17	2,83±0,82
8%	**2,09±0,97**	**2,48±1,08**	**2,04±0,86**

Considering 2 (two) as the boundary value between positive and negative, it is obvious from the results (table XIV) that none of the wines were really appreciated by the tasting panelists. The lower the values the more appreciated the wines, and no average was below 2.

4.4.2 Second Tasting

Based on the results from the triangular test on alcohol threshold perception obtained in the first tasting, in the second tasting the tasters panel was asked to compare sensorially two wines at a time, one untreated (normal alcohol content) and another partially dealcoholised (4% (v/v)). Making use of 5 sensorial descriptors commonly used to describe wines: fruity, body, bitter, sweet and sour, the panel was asked to evaluate each of them giving a value between '-5' (minus five, not good, under expected) and '+5' (five, very good, above expected) standing '0' (zero) for 'just right' (as expected).

In general, the panellists did not like either of the wines both in terms of aroma and flavour, being the average values for the attributes of fruity, body and sweet negative and for sour and bitter, even though the averages were positive (except for Riesling control and Dornfelder 4%), they were relatively low, all under 2.25. Despite the aroma and flavour changes expected during dealcoholisation there were no significant differences perceived between the control wine and the reduced alcohol one.

Fruitiness, even though in general considered not pleasant, in the whites with 4% (v/v) the panel members considered it not so unbalanced as in the control. The descriptors sour and bitter showed opposite patterns. Sourness values were higher in all 4% wines, this meaning that the control wine was considered more unpleasant. In all the wine pairs, the 4% wine samples were considered more unpleasant in terms of sweetness that the control wines. All wines lacked body, but curiously the white 4% wines were better evaluated than the control wines.

Looking at each pair individually, the results show no significant differences between the control and the 4% (v/v), respectively.

MD Cuvée Wines

The control MD Cuvée wine was 10.7% v/v and the panel perceived it as unpleasant and unbalanced in terms of fruitiness, body (sensation of fullness), and sweetness. This is, the wine was perceived as not enough fruity in the nose and lacking body and sweetness (Fig. 29). For both bitterness and sourness, despite the average being positive the values were really low (under 1).

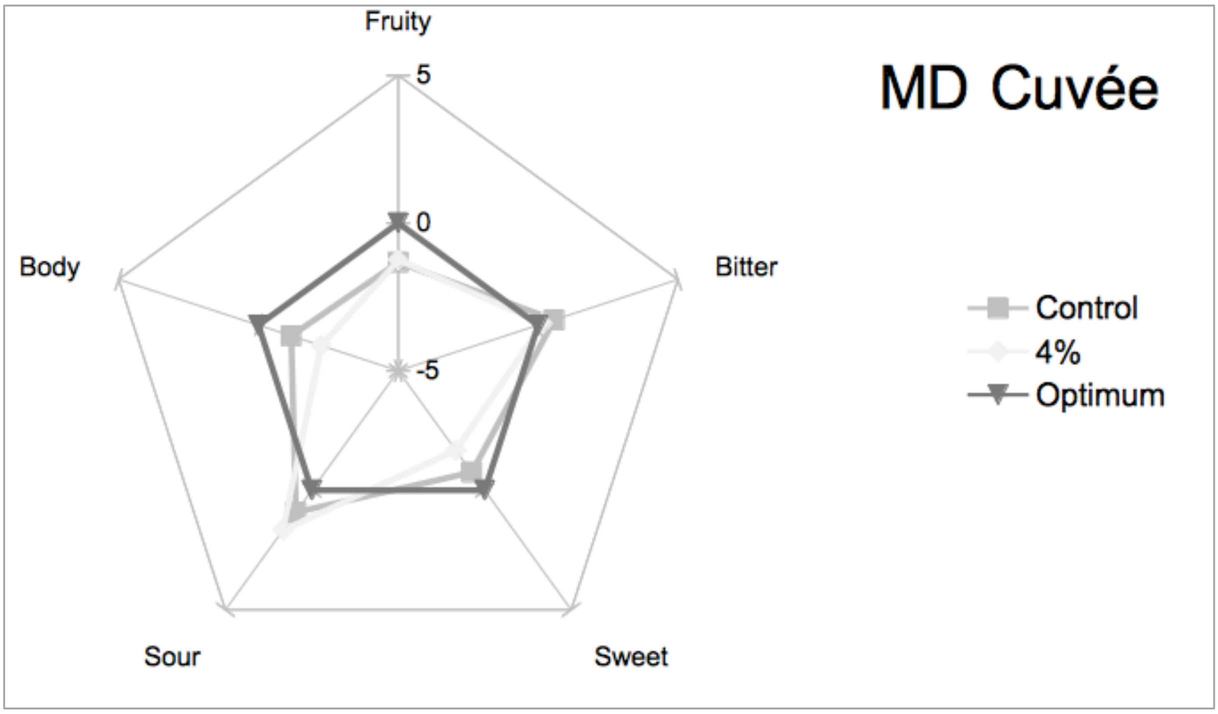


FIGURE 29. SENSORIAL ATTRIBUTES FOR MD CUVÉE WINE SAMPLES. (TASTERS N= 12).

The reduced alcohol wine with 4%, according to the panel of tasters had even less body and sweetness than the control, but fruity aroma perception was about the same. In the mouth, the wine was described as balanced in terms of bitterness and sourness (in this last descriptor, was considered better than the control wine).

Riesling Wines

The Riesling wines tasted were also not appreciated by the panel. The results showed that the reduced alcohol wine was perceived as more pleasant than the control wine (Fig. 30). Sour sensation was the best of the considered attributes (higher values), and the worse was the lack of body followed by a reduced fruity aroma, for both wines.

The control wine was considered more bitter (in negative terms) than the 4% one but at the same time sweeter.

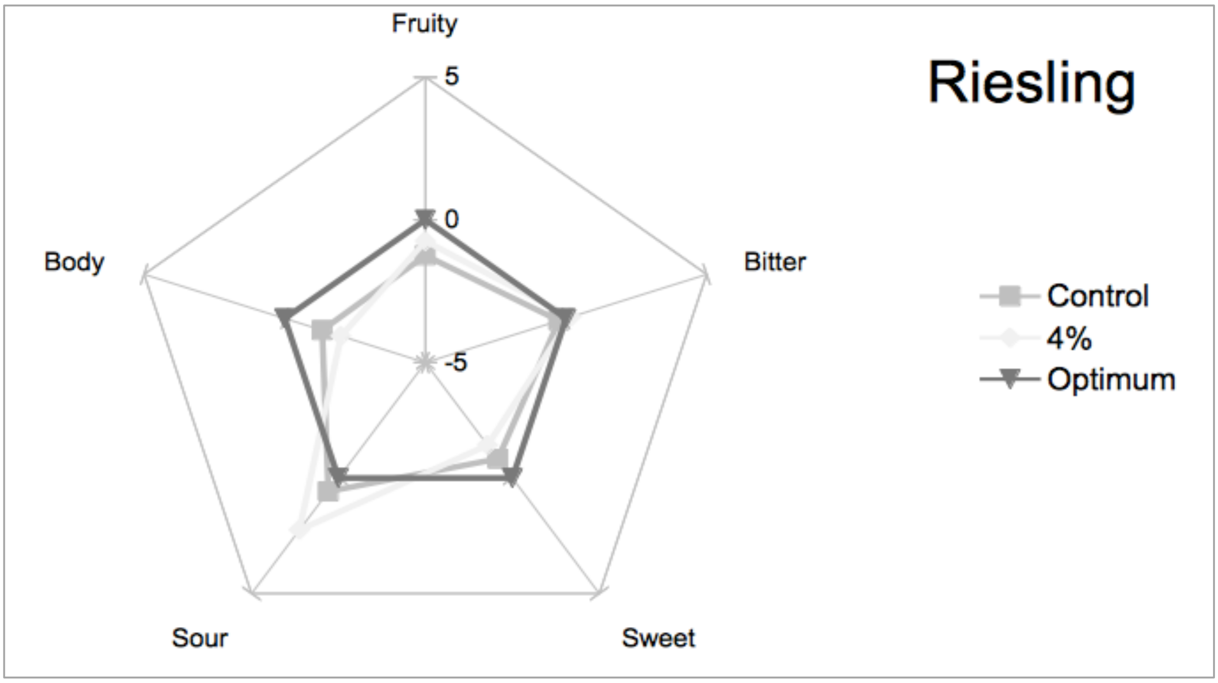


FIGURE 30. SENSORIAL ATTRIBUTES FOR RIESLING WINE SAMPLES. (TASTERS N= 12)

Dornfelder Wines

The two red wines were the least appreciated among the 3 pairs of tasted wines, in particular the 4% wine sample. Once again there's a reduction in fruitiness perception from the control to the reduced alcohol one, being stronger in this case than with the white wines (the 4% Dornfelder had the lowest value for fruity, -2,73, among all wines, Fig. 31). The same was observed for the body attribute, being the 4% Dornfelder considered by the panelists the one with less body and the most unpleasant for such descriptor.

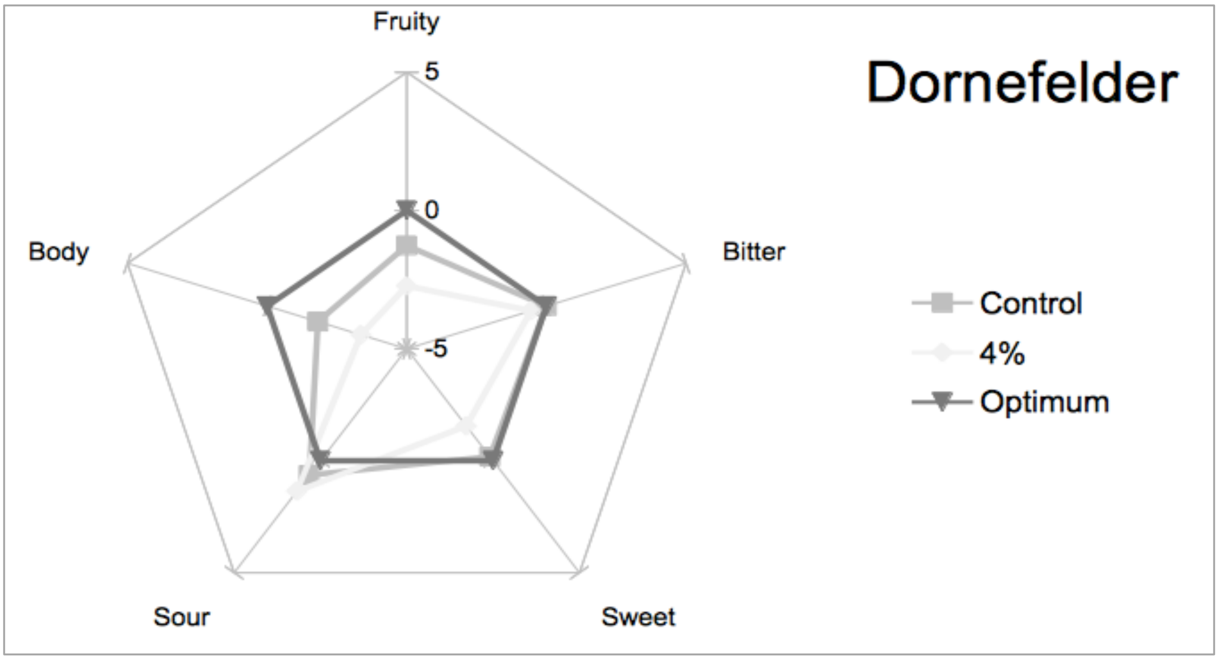


FIGURE 31. SENSORIAL ATTRIBUTES FOR DORNFELDER WINE SAMPLES. (TASTERS N= 11)

Chapter 5. Discussion

Reduced alcohol wines play currently an important role in the global wine market, but more and more they will become, I suspect, more common and accepted by the today-sceptics. Also, the more the experience of winemakers and wine researchers, the better the wine in the bottle. According to the wine critics and reviews and research scientific journals the main challenge is to produce reduced alcohol wines with comparable quality to a ‘normal alcohol content’ wine (or better).

There are now available, as described previously in the chapter 1, many different techniques and technologies to be applied both in the vineyard during the development and maturation of the grapes, and in the cellar before, during or after alcoholic fermentation (Pickering 2000, Schmidtke et al. 2012, Teissedre 2013). Most, if not all, of these strategies have an influence in the final characteristics of the wine. Anticipating the harvest date have implications in the chemical composition of the grape, namely in the sugar content - the main objective is to have a reduced sugar content leading to a reduced ethanol content after alcoholic fermentation, and in phenols and aroma precursors that develop during grape maturation – leading to, in principle lower concentrations and even different constitution. Reducing sugar content before or during alcoholic fermentation affects yeast activity and thus the metabolites produced during the alcoholic fermentation (AF) metabolism. These changes can (and do) affect aroma and flavour of the wine. Reducing ethanol level after the end of AF, though all fermentation products are present at the that time, some of them especially the more volatile will be, completely or partially, removed during the reduction/extraction method. Again, and it has been widely described, altering the final aroma and flavour of the wines.

Another important point is the legal framework of wines. According to the OIV and the EU legislation (OIV 18/73), a fermented grape beverage can only be considered as wine if the ethanol content is at least 8.5% v/v or more. Thus any ‘alcohol reduced wine’ is no longer, at least from a legal point of view, a wine. This has serious implications for the producers and for the consumers. Firstly, to sell a beverage produced as a wine but that cannot be called as such lacks the principal factor of attractiveness that is the name “WINE”. Secondly, from the consumer side looking for a wine, if one cannot find it in the right shelf of a store, most probably will not buy it. Lastly, if one wants to buy a wine than you need to call it so!!!

Within this context, and having in mind the available knowledge, this research project had a few but, in my opinion, crucial and pertinent objectives to move forward to solve the challenge of producing a Good Light Wine. First of all, we tested the perception threshold for alcohol reduction. This will allow to understand how far can one go in reducing ethanol level until it gets noticed. The result as implications in the winemaking process but it can also have in the legal framework. Second, we tested the sensorial opinion by subjecting a panel of experimented tasters to different wines - with normal and reduced alcohol content - to have an insight in what changes (are perceived), and what can and should be changed. This will allow to plan winemaking strategies. And thirdly, we tested alcohol reduction by osmotic distillation with red and white wines. This last point was more of a personal training to get the experience, the knowledge and the general overview of part of the process and the issues that it involves. The wines obtained will be used afterwards to continue the general project line, always looking forward to get a GOOD LIGHT WINE in the bottle in the near future!

5.1 Dealcoholisation of the wines by Osmotic Distillation

Two wines were used during the dealcoholisation trials to test for osmotic distillation technique. This technique based in membrane separation has been developed in a way that allows it to be used at room temperature and atmospheric pressure, producing no hazardous by-products. The studies published about its use to reduce ethanol content in wine are very positive and favourable to the process, referring a negligent loss of volatile compounds and a reduced influence in the final product (Schmidtke et al 2012, Schmitt et al. 2014).

Our trials were mostly to get the experience of using the machine and learning how to deal with practical issues, more than testing the resulting wines. This important step will, however, be done later to continue with the project. The two wines severely dealcoholised took about 2 days to be reduced by ~7% for a volume of 50 L of wine. This can be a limitation at an industrial scale, but surely larger equipment is to be available in the market to allow larger quantities of wine to be treated. A positive aspect of this technique is the absence of toxic by-products. The strip - aromatised and slightly alcoholised water - resulting from the process can possibly be re-used. Either as it is, a very light aromatised alcoholic summer drink - the so called Ready-To-Drink beverages (RTDs) or after total dealcoholisation as just an aromatised water.

During the dealcoholisation trials a red and a white wine were used. The white cuvée from unknown Italian grape varieties, was subject to physico-chemical analyses before and after dealcoholisation from 10.1% v/v (initial alcohol content) to 3.8% v/v (final alcohol content). It would have been interesting to run gas-chromatography or equivalent analyses to analyse aroma compounds present before and after the treatment. Such has been done already (e.g. Diban et al 2008, Schmitt et al. 2014) but, personally, this kind of analysis is crucial to better understand and improve the process. Also using the results of such analysis in comparison with results from a sensorial description by an experimented taster panel would very likely give important hints on how reduced alcohol wines can be improved.

Osmotic distillation seemed a straight forward technique to be used in a regular winery. It is time consuming - about almost 48 hours to reduce approximately 7% v/v of only 50 L of base wine.

5.2 Physicochemical analysis

The wines obtained from the local winery and to be used in the sensorial tastings were analysed. The 20 physico-chemical parameters measured allowed to better know the wines being used, however it is important to make the remark that each pair of untreated and dealcoholised wine could not be directly compared and conclusions about vacuum rectification alcohol reduction effect could not be taken because, unfortunately, the wines did not originate from the same original base wine. General trends can be pointed out, but in future trials this should be avoided, and wines originating from the same batch should be used.

When severely reducing ethanol content, some of the compounds increase due to concentration effect. That is the case, for example, of the total phenols. In all 3 wines the dealcoholised wine shows higher values for total phenols as well as total acidity.

From the colour analysis, it is very clear that the most affected wine is the red one, with reduced absorbance values in the severely dealcoholised wine. In the white wines it is the opposite with slightly higher values of absorbance in the dealcoholised wines. This can be explained by the retention of anthocyanins during the dealcoholisation process (which is method-dependent).

5.3 Microbial spoilage of “low alcohol” wines

At the time of bottling measured free sulphur dioxide was approximately, for all wines, 50 mg/L (or higher). The contaminations observed during the microbial spoilage trial all happened with the Spätburgunder 8% (five and seven weeks after bottling) and with the Dornfelder 8% after seven weeks. It is tempting to point the low alcohol content as a cause (lower anti-microbial protection), but the 4% alcohol content samples, in theory, more susceptible to microbial spoilage did not show any contamination.

One hypothesis is that during bottling, the 8% samples have been subject to an unknown source of contamination leading to the observed results. A second hypothesis, and in this case, also explaining the spoilage observed with the Dornfelder 8% is that the last sterility test was done in duplicate, and as it can be seen in figure 28, the petri dishes marked with an * were also used to remove a small sample for sulphur dioxide measurements. Even though the used material was clean, the procedure may have been a possible source of contamination: in both wines Spätburgunder and Dornfelder the contaminated samples were from those bottles.

However, and despite these events of observed microbial spoilage of the 8% samples, it seems that this level of sulphur protection is enough avoid spoilage. In what concerns legal aspects, this would mean that no alteration to the rules would need to be done as the current legal values are also efficient for such wines.

5.4 Sensorial analysis

Ethanol perception thresholds in wine have been tested before (e.g. Yu and Pickering 1998, Schmidtt et al. 2013) with discrepant results. Different wines lead to different thresholds but sensory tests found no significant differences between different techniques of alcohol reduction in the range of 2% v/v and the untreated control (Urbano et al. 2007, Bes et al. 2009, Lisanti et al. 2012) even when the panelists were trained wine professionals (Schmitt et al. 2013).

In our tastings as well, the taster panel was not able to tell apart wines with only 2% v/v difference in the alcohol content. From our results we could say that the perception threshold in alcohol content differences is about 4%, and in some cases for certain grape varieties can be even higher as we observed for the Riesling wines (also observed by Schmitt et al. 2013). Our results showed it was more difficult to the panel to differentiate between Riesling wines than between the other two tested wines. One hypothesis for this difference can be related to the strong aromatic character of this variety and other similar ones. The strong perceived aromas and flavours can mask the alcohol perception and thus affect the threshold.

Based on the tasting preferences expressed by the tasting panel in the second tasting, it was obvious that the wines, in general, were not appreciated. The red wine, Dornflder was the least enjoyed, which can mean that red wines may be more affected by dealcoholisation processes than the whites. Corroborating what is described is previous works, our panellists also described the reduced alcohol wines to be more acid and fruity than the control wines. As ethanol is removed, the volatility of the other aroma compounds is proportionally increased, giving the impression of a stronger fruity and floral character to the wines.

The sensorial evaluation of the control and reduced alcohol wines showed no significant differences between them. The lack of difference may result from the fact that the low ethanol wine was a blend between untreated and dealcoholised wine and not only resultant from dealcoholisation. The blending strategy to obtain a reduced alcohol wine may contribute to a recovery of the lost aromas and flavours, reducing the differences and improving the general wine quality.

Conclusions

Looking at the sum of all the obtained results and drawing out some conclusions takes us to important points that should be emphasised but it also leaves us with many other questions that can and should be explored sooner than later.

First, the legal limit of alcohol content in wines could be revised. If, even experienced tasters, cannot tell apart two wines differing in 2% v/v in their alcohol content, then the minimum value for the ethanol content in a wine could be changed by the OIV. Apparently, a wine with 8.5% cannot be told apart from a 6.5%. In a time of increased health concerns and global warming, one pushing ethanol levels down and the other pushing them up, respectively, this legal restriction needs to be discussed and better studied.

Where does this value came from? I searched for historical references but could not find any leading to the explanation.

Second, you cannot make a good grape wine from oranges. As mentioned by Aguera et al. (2010b) to produce a quality dealcoholised wine, despite the technique used, the most important is to choose an appropriate base must or wine. I think this was one of the big errors we may have committed during this project: to use low quality base wines. The sensorial analyses prove this by acknowledging a general depreciation of all the tasted wines, even the control ones.

Lastly, an important point to have into account in the future is to ensure that both control and dealcoholised wines come from the same original base wine. By doing so, we can analyse and compare physicochemically the wines but also run analytical aroma analysis to try to better understand the sensorial opinion expressed by the tasting panels. If it can be set a relationship between analytical compound analysis and sensorial opinion, much more easily winemaking strategies can be planned.

There is, in my opinion, a great potential to produce quality reduced alcohol wines, but it requires more technical and strategic work but also convincing strategies especially among the most traditional groups of the wine sector.

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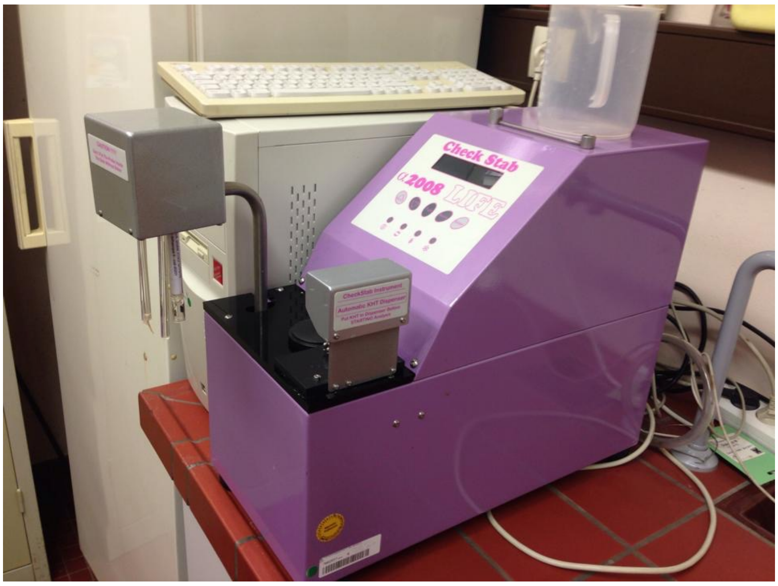
Appendixes

Appendix 1. Equipment

1. Ebuliometer used to measure alcohol content to the reduced alcohol wine.

2. Osmotic Distillation set-up

3. Spectrophotometer

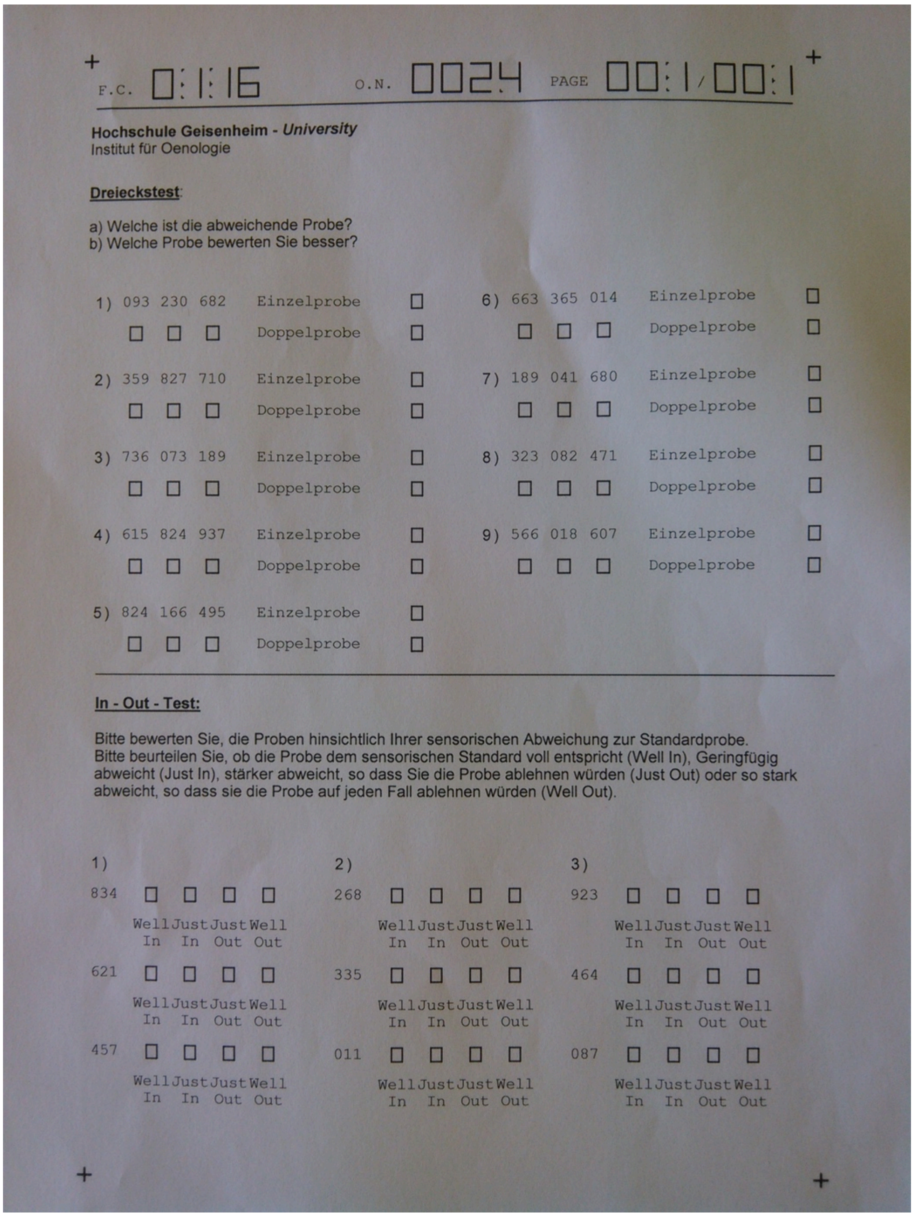


4. Check Stab ® for tartaric stability measurements.

5. Automatic Titrator to measure pH and Total Acidity.

Appendix 2. Tasting panels and Sensorial analysis sheets

1. Sensorial panels



F.C. 0:1:16 O.N. 0024 PAGE 00:1/00:1

Hochschule Geisenheim - *University*
Institut für Oenologie

Dreieckstest:

a) Welche ist die abweichende Probe?
b) Welche Probe bewerten Sie besser?

1) 093 230 682 Einzelprobe ☐
☐ ☐ ☐ Doppelprobe ☐

2) 359 827 710 Einzelprobe ☐
☐ ☐ ☐ Doppelprobe ☐

3) 736 073 189 Einzelprobe ☐
☐ ☐ ☐ Doppelprobe ☐

4) 615 824 937 Einzelprobe ☐
☐ ☐ ☐ Doppelprobe ☐

5) 824 166 495 Einzelprobe ☐
☐ ☐ ☐ Doppelprobe ☐

6) 663 365 014 Einzelprobe ☐
☐ ☐ ☐ Doppelprobe ☐

7) 189 041 680 Einzelprobe ☐
☐ ☐ ☐ Doppelprobe ☐

8) 323 082 471 Einzelprobe ☐
☐ ☐ ☐ Doppelprobe ☐

9) 566 018 607 Einzelprobe ☐
☐ ☐ ☐ Doppelprobe ☐

In - Out - Test:

Bitte bewerten Sie, die Proben hinsichtlich Ihrer sensorischen Abweichung zur Standardprobe.
Bitte beurteilen Sie, ob die Probe dem sensorischen Standard voll entspricht (Well In), Geringfügig abweicht (Just In), stärker abweicht, so dass Sie die Probe ablehnen würden (Just Out) oder so stark abweicht, so dass sie die Probe auf jeden Fall ablehnen würden (Well Out).

1)

	Well In	Just In	Just Out	Well Out
834	☐	☐	☐	☐
621	☐	☐	☐	☐
457	☐	☐	☐	☐

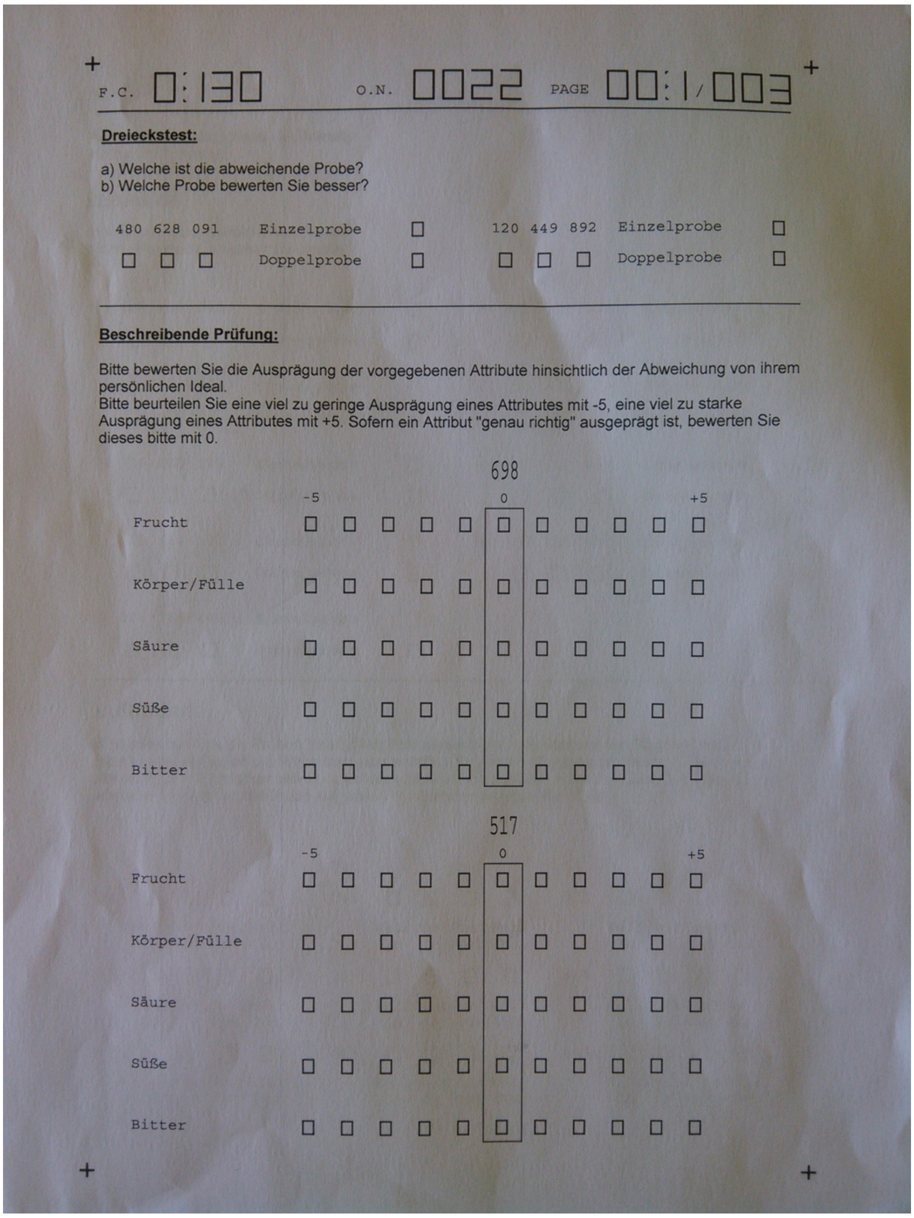
2)

	Well In	Just In	Just Out	Well Out
268	☐	☐	☐	☐
335	☐	☐	☐	☐
011	☐	☐	☐	☐

3)

	Well In	Just In	Just Out	Well Out
923	☐	☐	☐	☐
464	☐	☐	☐	☐
087	☐	☐	☐	☐

2. First tasting data sheet. Triangular and In-Out tests. (April 23rd, 2015)



F.C. 0:130 O.N. 0022 PAGE 00:1/003

Dreieckstest:

a) Welche ist die abweichende Probe?
b) Welche Probe bewerten Sie besser?

480	628	091			120	449	892		
☐	☐	☐	Einzelprobe	☐	☐	☐	☐	Einzelprobe	☐
			Doppelprobe	☐				Doppelprobe	☐

Beschreibende Prüfung:

Bitte bewerten Sie die Ausprägung der vorgegebenen Attribute hinsichtlich der Abweichung von ihrem persönlichen Ideal.
Bitte beurteilen Sie eine viel zu geringe Ausprägung eines Attributes mit -5, eine viel zu starke Ausprägung eines Attributes mit +5. Sofern ein Attribut "genau richtig" ausgeprägt ist, bewerten Sie dieses bitte mit 0.

698

	-5					0					+5
Frucht	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Körper/Fülle	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Säure	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Süße	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bitter	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

517

	-5					0					+5
Frucht	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Körper/Fülle	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Säure	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Süße	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bitter	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

3. Second tasting data sheet. Sensorial test. (May 3rd, 2015)