Master’s thesis 45 ECTS

Niki Alexi, ldq540

Characterization of aroma and flavour profile of red cultivars from Denmark

Volatile constituents, non-volatile composition and sensory properties of the Rondo, Regent, Cabernet Cortis and Bolero varieties

Academic advisor:

Mikael Agerlin Petersen, Department of Food Science, LIFE, KU

Bodil Helene Allesen-Holm, Department of Food Science, LIFE, KU

Submitted: 29/08/14
Preface
The present master thesis (45 ECTS credits) was written as final part of the master programme in Food Science & Technology, specialization sensory science at University of Copenhagen (KU), Faculty of Life Science, Denmark during the period of December 2013-August 2014.

Firstly I would like to thank my main supervisor Mikael Agerlin Petersen for his encouragement, support and feedback throughout the planning of the experiment and the writing process of the report. Furthermore I would like to thank Bodil Helene Allesen-Holm for jumping in as a co-supervisor when her expertise on sensory science was needed and for her invaluable guidance and support throughout the complete duration of the sensory science master.

Special thanks to Torben Bo Toldam-Andersen for his collaboration, useful suggestions through the initial planning of the experiment and the delivery of the wine samples used in this project.

Finally I would like to thank my co-worker Konstantina Sfyra for her invaluable help and collaboration throughout the project as well as the students who participated in the sensory panel for their help and interest in the project.

August 2014

Alexi, Niki (ldq540)
Characterization of aroma and flavour profile of red cultivars from Denmark

Abstract
The varietal aroma and flavour of four cultivars, Rondo, Regent, C. Cortis and Bolero, produced in the cool climate of Denmark as well as the effect of grape cultivation area was investigated using sensory evaluation (QDA) and instrumental analysis. The wines used to define the effect of «terroir» originated from two cultivation sites for each of the varieties in question. All wines were produced in 2011 by the same producer, under the same vinification conditions. For all samples the gross chemical composition (Volatile acidity, glycerol, fructose, glucose, tartaric acid, density, CO2, Folin C. Index, reducing sugar and total acid) was measured with NIR Spectrophotometer. Moreover DHS followed by GC-MS and GC-O were used for identifying the volatile compounds composition as well as the odour active constituents in the wines, respectively. Results from the instrumental analysis and sensory analysis were subsequently subjected to multivariate analysis (PLSR) to discover possible relationships between the two data sets. The results revealed that both grape variety and cultivation site played an important role in creating the overall sensory characteristics as well as the chemical composition of the wine cultivars. The Regent variety was characterized by a black pepper flavour and aroma mainly explained by the high concentration of terpenes and TDN in this samples. A high amount of esters contributed to the distinctive sensory quality of the C. Cortis variety. The Bolero cultivar developed, depending on the production site, a characteristic animal/leather flavour and aroma, which seems to derive mainly from acetaldehyde and tartaric acid concentrations. While Rondo appeared to have a balanced taste and flavour and its sensory quality was less dependent from the production site. Finally a correlation could be obtained between the levels of chemical constituents in the wines and their characteristic aroma and flavour.
# Table of contents

PREFACE .......................................................................................................................... 2

ABSTRACT ....................................................................................................................... 3

1. INTRODUCTION ......................................................................................................... 6

   1.1. Purpose of study ...................................................................................................... 6

2. BACKGROUND THEORY .............................................................................................. 7

   2.1. Wine production in Denmark .............................................................................. 7
       2.1.1. Climate conditions .......................................................................................... 7
       2.1.2. Grape varieties cultivated in Denmark ......................................................... 8

   2.2. Production and processing of red wine ............................................................... 8

   2.3. Aroma and flavour of wine ................................................................................. 9
       2.3.1. Varietal aroma and flavour .......................................................................... 10
       2.3.2. Formation of volatile compounds during vinification ................................ 11
               Formation of volatile compounds during fermentation .................................. 11
               Formation of volatile compounds during MLF and ageing ............................ 13
       2.3.3. Non-volatile constituents and wine flavour .................................................. 13

   2.4. Sensory evaluation .............................................................................................. 15

   2.5. Instrumental analysis .......................................................................................... 15
       2.5.1. Volatile compounds sampling methods-Dynamic Headspace Sampling (DHS) 16
       2.5.2. Gas Chromatography (GC) ......................................................................... 16
       2.5.3. Gas Chromatography coupled to Mass spectrometry (GC-MS) .................. 17
       2.5.4. Gas Chromatografy- Olfactometry (GC-O) ............................................... 17
       2.5.5. Near-Infrared spectroscopy (NIR) ............................................................... 18

   2.6. Data analysis ....................................................................................................... 19
       2.6.1. Quality control of sensory profile data ......................................................... 19
       2.6.2. Multivariate analysis in Sensometrics and Chemometrics ....................... 19

3. MATERIALS AND METHODS .................................................................................... 20

   3.1. Experimental Design and wine samples .............................................................. 20
       3.1.1. Vinification process ...................................................................................... 20

   3.2. Sensory evaluation ............................................................................................. 20
       3.2.1. Pre-study ...................................................................................................... 21
       3.2.2. Preparation for the sensory analysis ............................................................. 21
       3.2.3. Sensory vocabulary development, training and profiling ........................... 21

   3.3. Chemical analyses ............................................................................................. 22
       3.3.1. GC-MS analysis ......................................................................................... 23
       3.3.2. GC-O analysis ............................................................................................ 23
3.3.3. Wine scanner analysis ........................................................................................................... 23

3.4. Data analysis ............................................................................................................................... 24
3.4.1. Pre-processing of data ............................................................................................................ 24
3.4.2. Sensory data .......................................................................................................................... 24
3.4.3. GC-MS data ........................................................................................................................... 25
3.4.4. GCO data .............................................................................................................................. 25
3.4.5. Wine scanner data .................................................................................................................. 26
3.4.6. Linking sensory to chemical data ........................................................................................... 26

4. RESULTS ........................................................................................................................................ 27
4.1. Sensory analysis ........................................................................................................................... 27
4.1.1. Panel performance .................................................................................................................. 27
4.1.1. Sensory profile of wines .......................................................................................................... 28

4.2. Chemical analyses ....................................................................................................................... 31
4.2.1. Aroma analysis-GCMS ......................................................................................................... 31
4.2.2. Aroma analysis-GCO ............................................................................................................. 34
4.2.3. Wine scanner analysis ............................................................................................................ 36

4.3. Linking sensory to chemical data ............................................................................................... 37

5. DISCUSSION .................................................................................................................................... 39
5.1. Sensory analysis ........................................................................................................................... 39
5.1.1. Panel performance .................................................................................................................. 39
5.1.2. Sensory profile of wines ......................................................................................................... 40

5.2. Chemical analyses ....................................................................................................................... 41
5.2.1. Aroma analysis-GCMS & GCO .............................................................................................. 41
5.2.2. Wine scanner analysis ............................................................................................................ 44

5.3. Linking sensory to chemical data ............................................................................................... 46

6. CONCLUSION ................................................................................................................................. 49

REFERENCES ...................................................................................................................................... 51
1. Introduction
Denmark was accepted as a wine producing country by the EU commission in 2000. The transformation of Denmark into a wine producing country was mainly facilitated by two factors, improvement of climate conditions for grape cultivation as well as introduction of new grape varieties more suitable for production of wine in cool climate regions (Bentzen & Smith, 2009).

Rondo and Regent cultivars constitute the first and the second most cultivated varieties in Denmark respectively (Becker & Toldam-Andersen, 2012). Moreover other grape cultivars such as Cabernet cortis and Bolero show optimal characteristics for production of red wines in Denmark’s cool climate area. While numerous studies have been conducted to characterise the chemical composition of wines made from different traditional varieties, limited scientific literature exist on the aroma and flavour characteristics of monovarietal red wines produced in Denmark.

While it has been found that the general volatile composition of wines among cultivars is quite similar, the varietal aroma can be contributed to the variances in the ratios of chemical constituents as well as the existence of specific impact compounds for a number of cultivars (Styger et al., 2011). Moreover the final sensory quality of wine can originate from the balance of the volatile and non-volatile groups. Several variables can affect the aforementioned groups of constituents and therefore play a role in final wine quality. Therefore characterisation of aroma and flavour of wines originating from a specific grape cultivar requires information about the composition of the volatile and non-volatile flavour components as well as their contribution to the sensory properties of the wine. To achieve this purpose sensory evaluation (QDA) as well as chemical analysis (GC-MS, GC-O, NIR spectroscopy) are commonly employed.

1.1. Purpose of study
The aim of this study was to define an initial sensory profile and overall chemical composition of monovarietal red wines produced in Denmark including Rondo, Regent, Cabernet Cortis and Bolero as well the effect the area of production has on the quality of the wines. In order to investigate the aroma and flavour quality of each of the varieties in question two variables were included in the design of the study; grape variety and «terroir». While the effect of other variables such as vinification process (same producer and vinification process for all wines included in this study) and year of production (same year 2011) was limited.
2. Background theory

2.1. Wine production in Denmark

Denmark became officially a wine producing country in the year 2000 when a revision of the EU Wine Regulation made the commercial production of wine legal for Denmark. More specifically from August 2000 Danish wine producers were permitted to produce, on a commercial basis, wine without geographical origin on the label. Another change came in 2007 when Denmark’s request of producing wine with a geographical indication on the label was accepted by the EU commission. This change meant that commercial wine producers could now include year of harvest, grape variety as well as region (‘regional wines’ from Jutland, Funen, Zealand and Bornholm) on the label (Bentzen & Smith, 2009).

Since the formal approval by the EU in 2000, Danish wine production has increased rapidly with the Danish Vineyards Association counting over 1400 members in 2012. Moreover, the commercial wine producers reached the number of 90 at the same year (Becker & Toldam-Andersen, 2012). The Danish commercial production of wine follows the same EU regulations as other wine producing countries. Some exceptions to the general EU rules exist with the most important being the use of saccharose which is vital for Danish wine production due to the region’s climatic conditions (Bentzen & Smith, 2009).

Among others two were the major factors that allowed Denmark to be transformed to a wine producing country; more optimal climate conditions for grape cultivation compared to past one’s, and development of new grape varieties more suitable for production of wine in cool climate regions (Bentzen & Smith, 2009).

2.1.1. Climate conditions

Climatic conditions are crucial when dealing with wine production. Denmark belongs to the northern regions of the EU and is characterised as a cool climate region. The weather conditions the northern parts of Europe have made commercial production of wine difficult in the past.

However, recent climate changes, which include milder winter conditions, smaller chances of late spring frost as well as more optimal weather conditions in the August-September period, have made the weather conditions more appropriate for grape cultivation (Bentzen & Smith, 2009). Still, tolerance to late spring frost and adaptation of grape cultivars to the climate is important for cultivation in this cool climate area (Gustafsson & Mårtensson, 2005). Moreover, due the humid climate of Denmark and the fact that a limited number of fungicides is permitted in grape cultivation, strong tolerance against infections is also crucial for the development of the grapes.

It is important to underline that weather conditions in Denmark show a significant variation from both year to year and region to region. Therefore location of the wine fields is very important. While the climate varies considerably between regions wine producers are distributed among all areas of Denmark (Bentzen & Smith, 2009). Therefore in order to support the development of grapes special cultivars that can withstand the variability of the climate as well as the general weather conditions should be used.
2.1.2. Grape varieties cultivated in Denmark

Traditional grape varieties are not suitable for producing wine in Denmark; the grapes are not able to mature due to the country’s climatic conditions (Bentzen & Smith, 2009). The grape varieties which can prosper in Denmark should be able to withstand the cold climate and have a short growing season in order to be suitable for wine production. The introduction of new grape varieties that have a short ripening period and can withstand the cold climate of the region has allowed northern regions like Scandinavia to produce wine with sufficient oenological capabilities (Gustafsson & Mårtensson, 2005).

Among the varieties used for the production of red wine, Rondo has become the most dominantly cultivated in Denmark. Rondo is used for the production of single grape red wine but also comprises the basic component in blends (Bentzen & Smith, 2009). Rondo is an interspecific variety, originated from Germany, which has a short ripening period and is quite resistant to fungus infections (Schwab et al., 2000). Regent it is constitutes the second most cultivated variety in Denmark and is a again an interspecific, fungus resistant cultivar which originated from Germany (Schwab et al., 2000). Some other varieties, which are cultivated to a smaller extend, but are suitable for producing red wine in the cold climate of Denmark, are Cabernet Cortis and Bolero. Cabernet Cortis is also an interspecific variety originated from Germany and was the 4th most cultivated red wine variety in 2012, while Bolero constituted only 1% of the total vines in the same year (Becker & Toldam-Andersen, 2012).

While previous research has focused on characterizing the chemical composition and aroma quality of more traditional wine cultivars, no extensive scientific literature exists on the aroma and flavour of wine varieties produced from the “cold climate” grapes utilised in Denmark such as of Rondo, Regent, Bolero and Cabernet Cortis wines. Some studies exist on more practical aspects of the Rondo grape such as sugar accumulation, acid content and growing degree days needed for this cultivar (Moulton et al.; Schwab et al., 2000). Moreover, in a study by Antoce and Namolosanu (2011) it was shown, by the use of electronic nose, that Rondo wine has a distinct aroma profile, which is significantly different from other wine varieties like Merlot, but the characteristics of the aroma of the wine were not given.

2.2. Production and processing of red wine

There are two pre-fermentive steps that usually take place in the production of red wine; Crushing/destemming and Maceration. Opposite to white wines, skins and seeds are not removed but stay in contact with the grape juice after crushing, in a process called maceration. Maceration can take place both before and during fermentation (Bakker & Clarke, 2011). The process of maceration is important in red wines since it can increase the level of extraction of several flavour compounds which can strongly determine the final wine style (Polášková et al., 2008; Bakker & Clarke, 2011). The type and extend of the maceration can vary according to the desired final quality of the wine, still it has been shown that the result of the extraction is more depended on the initial composition of the compounds in the grapes rather than the choice of extraction method (Bakker & Clarke, 2011).

Frequently ‘punch downs’ (cap of fermentation pushed down by physical punching) or ‘pump overs’ (removing must from the bottom of the fermentation vessel and spraying it over the top to wet the cap) take place in red wine-making. This action takes place in order to reduce the solids on the top of the
fermentation vessel which are gathered there due to carbon dioxide formation during fermentation (Bakker & Clarke, 2011).

Two main fermentation steps occur in red wine making. The first fermentation is the alcoholic fermentation, the yeast strain used in this step is crucial in the resulting wine quality and composition. Alcoholic fermentation is the main process which causes the transformation of must into wine. The yeast stain used in this process can vary with the most important being of the *Saccharomyces cerevisiae* species; still several other kinds of yeast may be involved (Bakker & Clarke, 2011). A current trend in wine making is the introduction of the use of mixed starter cultures in the fermentation process. Mixed starter cultures are composed by one or more non-*Saccharomyces* yeast strains as well as an industrial *S. cerevisiae* wine yeast which can add more complexity to the resulting aroma and flavour of wine (Styger et al., 2011). Maceration can continue throughout the whole period of fermentation or grapes may spend only 2–3 days on the skins. In case fermentation needs to continue without maceration, the juice can be separated from the skins, which results to lighter red wines. In the presence of skins the fermentation temperature varies between 24–28°C (no higher than 30°C) for up to two weeks which results to the production of traditional red wines (Bakker & Clarke, 2011).

Following alcoholic fermentation, some wines can undergo a secondary fermentation known as malolactic fermentation (MLF). MLF is commonly practiced in red wines, and especially desirable in high acid red wines produced in cool climate regions which benefit from conversion of the sharp tasting dicarboxylic L-malic acid to the monocarboxylic L-lactic acid and carbon dioxide (deacidification of wine) (Bakker & Clarke, 2011; Styger et al., 2011). This process is a result of lactic acid bacteria including the *Oenococcus oeni*, *Lactobacillus spp., Leuconostoc spp., and Pediococcus spp* (Styger et al., 2011). The lactic acid bacteria of malolactic fermentation can occur naturally or added in with a specially prepared inoculum (Bakker & Clarke, 2011). Moreover it has been shown that MLF not only can enhance the volatile composition of wine but also can play a role in its stabilization (Styger et al., 2011).

One of the most important post-fermentive steps is maturation of wine. Red wines are often sold after some ageing. As a general rule it is believed that the darker the wine (high tannin content) the more it can benefit from the maturation process when compared to lighter wines (low tannin content). The maturation process can be achieved by barrel ageing or by bottle ageing. Barrel ageing is often conducted in small oak barrels and is usually combined with maturation in large stainless steel vats. This process can last from three months up to about three years and is followed by bottle storage. Bottle ageing is a common maturation practise and can affect the quality of some wines (distinct chemical changes occur in red wines when ageing in-bottle). After the finalization of wine, storage takes place in relatively low storage temperature while sulfur dioxide concentrations are kept low during the whole process (Bakker & Clarke, 2011).

### 2.3. Aroma and flavour of wine

The perceived aroma and flavour of wine is the result of a multitude of interactions between all chemical constituents within it (volatile as well as non-volatile), sensory receptors as well as other factors such as wine’s serving temperature. All the aforementioned factors can change the perceived quality of wine in a highly complex manner (Styger et al., 2011).
However wine odorants are considered the major factor affecting the final aroma and flavour of wine (Polášková et al., 2008). In order to categorise the effect the odorants have on the final wine aroma the notion of odour activity value (OAV) is used. OAV equals the concentration of aroma compound in the wine divided by its detection threshold (the concentration at which the aroma compound is firstly perceived by the sensory receptors when diluted in wine/water). High impact odorants are considered those which have high OAV and/or concentrations and can therefore have an extensive impact on the final aroma and flavour of wine (Polášková et al., 2008; Styger et al., 2011). However, when aroma compounds co-exist in a mixture they can interact by supressing or enhancing each other and as a result variations in the concentration of low impact odorants can also affect the aroma and flavour of wine. The final wine quality is determined by the multitude of those unique and non-linear interactions (Styger et al., 2011).

Finally, great variations in the sensory perception of wine can occur even by more simple factors such as the serving temperature of wine or even the shape of the wine glass (Molina et al., 2007). As a result the aroma bouquet and the sensory perception of wine is a highly complex and subjective process, which depends on many different factors besides its chemical composition.

2.3.1. Varietal aroma and flavour

Most grape cultivars have a distinct aroma and flavour profile that is referred to as varietal flavour and aroma. While amongst the different cultivars the overall volatile composition is very similar, the distinctive profile of each variety can be mostly related to minor variations in the ratios of a large number of compounds, rather than to individual compounds of the wine (Polášková et al., 2008; Styger et al., 2011).

There are several families of impact odorants that can be associated with distinctive grape cultivars. One important family of impact odourants is the terpene one. The initial synthesis of terpenes is believed to occur in grape berries (Bakker & Clarke, 2011). From this family sesquiterpenes and monoterpenes, which derive from the precursor mevalonate, contribute to the varietal floral aroma of grapes and wines of the Muscat, Riesling and Shiraz cultivar (Bakker & Clarke, 2011; Styger et al., 2011). The most important representatives of this group are linalool, geraniol, nerol, and citronellol (Ebeler, 2001; Ribéreau-Gayon et al., 2006; Styger et al., 2011). These compounds can be found in two forms; the non-volatile glycosides, which are odourless, and the free form, which has lost its sugar attachment and is odour active (Bakker & Clarke, 2011; Styger et al., 2011). The ratio between the two forms varies; depending on the ripening stage of the berry, with overripe berries having a bigger content of bound forms compared to free ones, as well as between grape varieties (Bakker & Clarke, 2011; Styger et al., 2011). While free terpenes are not affected by the fermentation process the bound forms can be hydrolysed by chemical and enzymatic reactions (yeast glucosidases) during fermentation resulting to an increased ratio of the free volatile form (Ebeler, 2001; Bakker & Clarke, 2011; Styger et al., 2011). Finally, it is important to mention that terpenes tend to have floral aromas and while they can be found in wines in small concentration compared to their threshold values the impact they have on the aroma of the wine is synergistic (Bakker & Clarke, 2011; Styger et al., 2011).

Rotundone is a recently identified sesquiterpene, which can act as an impact compound conferring a distinctive white/black peppery aroma to specific wine varieties. Recent discoveries revealed that rotundone was responsible for the peppery aroma and flavour in Shiraz grapes and wines. The varietal
aroma of this cultivar was in past attributed to complex interactions of many odorants, or to piperine and related alkaloids, which impart ‘heat’ in the mouth (Styger et al., 2011).

C13-norisoprenoids are a group of compounds, which derive from carotenoid degradation during grape ripening and can also play a role in varietal aroma (Ebeler, 2001; Ribéreau-Gayon et al., 2006; Styger et al., 2011). The oxidation that takes place during ripening produces odour active fragments which can contribute to complex aromas (tea, lime, honey etc.) in several varieties of wines such as Syrah, and Cabernet Sauvignon (Ebeler, 2001). The main representatives are b-ionone (viola aroma), b-damascenone (exotic fruits), b-damascone (rose) and b-ionol (fruits and flowers) (Styger et al., 2011). C13-norisoprenoids derivatives Vitispirane (eucalyptus) and TDN/ 1, 1, 6-trimethyl-1, 2-dihydronaphthalene (kerosene) belong to this group (Ebeler, 2001). Similarly to monoterpenes, C13-norisoprenoids can be found in both free and bound form and are mainly found in grapes and wines in the form of the non-volatile glycosidically bound precursor (Ebeler, 2001; Ribéreau-Gayon et al., 2006). The same pattern of variations in concentrations is also followed during ripering and exposure of grapes to sunlight; decrease in carotenoids and increase, mainly in glycosylated forms of C13-norisoprenoids derivatives (Ribéreau-Gayon et al., 2006).

While varietal volatiles play an important role in flavour and aroma of wine the majority of the components of finished wine derive not from the grapes itself but rather from primary or secondary metabolic processes during fermentation (Styger et al., 2011).

2.3.2. Formation of volatile compounds during vinification

This section will mainly focus on the major volatile constituents of wine which derive from the vinification process (alcoholic fermentation, MLF, aging) and their effect on final wine quality. Yeast selection and fermentation is the first important step of the wine making process. According to Fleet (2003) the yeast selected for fermentation can influence the aroma quality through several mechanisms with the most important being de novo biosynthesis of aroma compounds. Moreover, fermentation can also affect the levels of varietal aroma compounds, discussed in paragraph 2.3.1 directly or through influencing the concentration of their precursor molecules (Styger et al., 2011).

Formation of volatile compounds during fermentation

The volatile compounds, which derive from yeast fermentation, constitute the largest percentage of volatiles in terms of numbers (Ebeler, 2001; Polášková et al., 2008; Styger et al., 2011). Still, the individual impact fermentation derived volatiles have on final wine quality can be quite small due to the low OAVs in finished wine (Ebeler, 2001; Polášková et al., 2008; Styger et al., 2011). Though due to the synergistic or antagonistic relationships of some compounds, the effect the metabolites of fermentation can have on aroma quality combined can influence and establish wine aroma in a considerable extend (Polášková et al., 2008; Styger et al., 2011).

Ethanol is a by-product of glycolysis during primary fermentation and a major component of wine (Ebeler, 2001; Styger et al., 2011). Therefore the concentration of ethanol in wine depends on initial sugar (glucose and fructose) concentration of the grape juice as well as the vinification procedure (Ebeler, 2001; Bakker & Clarke, 2011). Although ethanol has a low OAV it can have a great influence on wine quality due to its high concentration and its influence on other group of compounds which define wine’s perceived aroma and flavour (Ebeler, 2001; Styger et al., 2011). It has been shown that the reduction of ethanol from 9% to 7%
induced an increased perception of fruity, flowery, and acid flavours and aromas of the wine (Grosch, 2001). Moreover, increases in ethanol’s levels enhanced the perceived astringency as well as bitterness (due to polyphenols) and hotness of resulting wine (Ebeler, 2001; Styger et al., 2011). It should be noted that the extraction of several compounds, including terpenes, is facilitated by the production of alcohol during fermentation (Bakker & Clarke, 2011).

Acetaldehyde is also a compound formed by primary yeast metabolism and acts as a precursor molecule for synthesis of several other molecules including ethanol and diacetyl. The aroma of this compound depends greatly on its concentration, with low levels releasing a pleasant fruity aroma to wine which is converted to a pungent irritating odor reminiscent of green grass or apples when it reaches higher ones (Styger et al., 2011). Therefore, high concentrations of this compound can affect the sensory quality of wine in a negative way (Polášková et al., 2008).

Besides the primary metabolites, discussed above, secondary metabolites, not directly related to the central metabolism pathway, are also synthesized during yeast fermentation. These compounds can be synthesized by the metabolism of amino acids or fatty acids. The pathway in which amino acids are catabolized into higher alcohols is referred to as Ehrlich reaction; still this reaction explains partly the catabolism of amino acids to volatile compounds. Among this group, higher alcohols (fusel alcohols) as well as their corresponding esters and volatile acids are considered quite important when referring to aroma and flavour of wine. This is supported by the fact that differences in the volatile compounds, and therefore aroma quality, of certain wine varieties can be attributed, to some extent, to variations of the free amino acids present in the grape and must (Bakker & Clarke, 2011; Styger et al., 2011).

Fusel alcohols present in wines predominantly include C3-C5 strait chain, branched n-alcohols as well as 2-phenylethanol (Polášková et al., 2008). Among this group 3-methylbutanol, 2-methylpropanol and propanol have been found to be major constituents of wines (Ebeler, 2001). On the other hand, in terms of aroma 2-methyl butanol and 2-phenylethanol seem to be the more odiferous compounds since the majority of the remaining fusel alcohols are usually found in concentrations below their detection threshold (Ebeler, 2001). As a family, fusel alcohols, when they are found in high concentrations, contribute negatively to wine’s sensory quality (Ebeler, 2001).

Volatile esters is one of the most important classes of aroma compounds when referring to wine flavour and aroma (Bakker & Clarke, 2011). The majority of esters found in wine are acetate esters as well as ethyl esters of fatty acids, which are mainly formed during fermentation by lipid and acetyl-CoA metabolism (Ebeler, 2001; Bakker & Clarke, 2011; Styger et al., 2011). Ethyl esters (such as ethyl butanoate, ethyl hexanoate, ethyl octanoate) are intermediates of fatty acid metabolism and are found in lower concentration when compared to acetate esters (Bakker & Clarke, 2011). Acetate esters (such as isoamyl acetate, propyl acetate, hexyl acetate, phenethyl acetate) are products of the reaction of acetyl-CoA with alcohols formed due to degradation of amino acids, carbohydrates, and lipids. Esters do not only derive due to enzymatic reactions but are also released from the yeast into the wine (Bakker & Clarke, 2011). The ester compounds predominantly found in wine are ethyl acetate and isoamylacetate (Polášková et al., 2008; Bakker & Clarke, 2011). 3-methylbutyl acetate, while found in lower concentrations, seems to be one of the most important esters found in wines due to the compounds high OAV (Ebeler, 2001). The ester family is
responsible for fruity aromas perceived in wine. More specifically, it is known that lower aliphatic ethyl esters give fruity characteristics of different kinds (mostly tropical tree fruit, banana, pineapple) up to about ethyl heptanoate, whereas the higher esters release more soapy, oily and candle wax notes (Bakker & Clarke, 2011).

Moreover some compound which can create faults in wines like acetic acid, also called volatile acid, can be created as by-products of the original fermentation procedure Acetic acid is a volatile organic acid by-product of yeast or bacterial metabolism which attaches a Vinegar-like odour/flavour and it can have an undesirable effect in wines when found in high concentrations (Bakker & Clarke, 2011; Sáenz-Navajas et al., 2012).

**Formation of volatile compounds during MLF and ageing**

A second step that many wines, especially high acid ones, undergo during the vinification process is MLF. MLF can affect wine aroma and flavour quality through the production of new volatile metabolites as well as modification of existing ones derived from grapes and fermentation (Ebeler, 2001; Polášková et al., 2008; Styger et al., 2011). In terms of wine aroma the most important effect MLF has is the production of diacetyl (2,3-butanedione), a constituent of wine formed from acetaldehyde by lactic acid bacteria (Ebeler, 2001; Polášková et al., 2008; Styger et al., 2011). Diacetyl is an odour active volatile compound (high OAV) which contributes yeasty, nutty, toasty notes to wine when found in low concentrations (Ebeler, 2001; Styger et al., 2011). However, when found in high concentrations it gives a characteristic buttery flavour to wines (Ebeler, 2001; Polášková et al., 2008; Styger et al., 2011). Due to its highly reactive nature it can react with other wine constituents (cysteine) further influencing wine aroma (Styger et al., 2011). During the vinification process the major concentration diacetyl is transformed to acetoin and 2,3-butanediol. Acetoin and 2,3-butanediol are metabolites of diacetyl, which do not have high OAV and therefore do not contribute significantly to the aroma of finished wine.

The general effect MLF has on wine aroma is enhancement of the fruity notes, due to the formation of esters, and buttery notes, due to formation of diacetyl. Moreover it has been show that wines that undergo MLF can show a reduction on vegetative, green/grassy aromas due to the catabolism of acetaldehyde by lactic acid bacteria. Moreover, enzymes produced by lactic acid bacteria can release grape-derived aroma compounds from their glycosylated form making them volatile, thus aroma active (Styger et al., 2011).

Changes in the aroma and flavour quality of wine can also occur during ageing and maturation. The wine aging process results to modification and loss of characteristic wine aromas derived from the grape variety and fermentation process. This leads to wines, of the same or different varieties, growing more similar profile during maturation and gaining an aroma more characteristic of older wines often referred to as bottle aged bouquet (Bakker & Clarke, 2011; Styger et al., 2011). Moreover, with respect to the aging carriers, aging in barrels can add to the richness and complexity of wines through simple extraction of aroma constituents from the wood to the wine (Styger et al., 2011).

### 2.3.3. Non-volatile constituents and wine flavour

According to Breslin (2001) non-volatile constituents, which are majorly responsible for taste and tactile sensations, play an important role in flavour by creating the sensory foundation on which the volatile components build. On one hand, the non-volatile matrix affects the release of volatile compounds, and
therefore the aroma and flavour quality of wine, while on the other hand the sensory characteristics attributed to non-volatiles are not fully realised in the absence of volatile constituents (Sáenz-Navajas et al., 2012). Thus, the overall perception of wine’s flavour and aroma is highly depended on both volatile and non-volatile constituents as well the balance between them.

Concerning wine’s sweetness, it is mainly attributed to the presence of the reducing sugars glucose and fructose. The perceived sweetness can also be affected by the wine’s content in ethanol and glycerol. Glycerol is the most common liquid fermentation product after ethanol and is formed during the transformation of sugar to alcohol during fermentation (Bakker & Clarke, 2011; Styger et al., 2011). Glycerol is a compound with a sweet taste but also a high sensory threshold, thus it can affect sweetness by itself to a small extend (Bakker & Clarke, 2011). With respect to the effect glycerol has on the general sweetness in wines, it has been shown that in some cases, depending on concentration and grape variety, it enhances the effect of perceived sweetness in both white and dry red wines (Styger et al., 2011; Sáenz-Navajas et al., 2012). Still, the extend of the contribution of this compound to sweetness is not clear (Bakker & Clarke, 2011). Moreover a study by Nieuwoudt et al. (2002) suggested that while the concentration of glycerol can have a significant effect on the perceived white wine quality, no statistical relationship can be proven between glycerol concentration and quality of red wine. Finally it is important to mention that a cognitive interaction taste aroma interaction between perceived sweetness and fruitiness exists, with the increase in fruity quality inducing an enhancement of the perceived sweetness in wines and vice versa (Zamora & Guirao, 2002).

Perceived sourness is highly depended on wine’s acid content. The main non-volatile acids (tartaric, malic and citric) are already present in the grape, still there are also acids mainly derived by fermentation process (lactic acid and succinic acid) (Bakker & Clarke, 2011). The perception of sour taste in wine has been shown to enhance with increased levels of titratable or total acid, while the opposite relationship exists with pH values (Sáenz-Navajas et al., 2012). Moreover sour taste is mainly attributed to non-volatile acids, which account approximately for 90% of the total acidity in wine taste, while volatile ones (such as acetic acid) also play a role to wine’s odour/aroma (Bakker & Clarke, 2011). As a general rule, insufficient concentration of organic acids results to flat tasting wines, while excess concentration of the same group can induce an unpleasant acidity, suppress other desirable flavours and negatively affect the general mouthfeel of wine (Biasoto et al., 2010; Bakker & Clarke, 2011; Sáenz-Navajas et al., 2012). Balanced acidity can modify the sourness sensation to a pleasant freshness as well as reduce the sweetness levels of wine (Biasoto et al., 2010; Bakker & Clarke, 2011; Sáenz-Navajas et al., 2012). With respect to the sensory quality of the individual organic acids; tartaric acid is the most abundant and can induce a sharp unpleasant taste to wine especially when found in high concentrations (> 5 g L-1) (Biasoto et al., 2010; Sáenz-Navajas et al., 2012). Malic acid induces a harsh, green taste to wine which is gradually lost during MLF, where it is transformed into lactic acid, which has a mild flavour (Biasoto et al., 2010; Bakker & Clarke, 2011).

Phenolic compound with low molecular weights, monomeric flavonoids: myricetin, quercetin and catechin, are related to the bitter sensation of wines (Biasoto et al., 2010; Sáenz-Navajas et al., 2012). Besides the constituents which create the bitter sensation, the perceived bitterness can also be enhanced by the presence of other constituents such as ethanol and by an increase in the pH value of wine (Sáenz-Navajas et al., 2012).
et al., 2012). Concerning taste to taste interactions, the perceived bitterness sensation seems to be suppressed in the presence of sugars or glycerol (sweetness) (Sáenz-Navajas et al., 2012).

Astringency is a dry, puckering mouthfeel caused by wine consumption (especially red) and has been described as a sensation caused by multiple perceptual phenomena (Sáenz-Navajas et al., 2012). With respect to the constituents causing astringency, tannins and more specifically procyanidins seem to be the main contributors of astringent sensation in wines (Monagas et al., 2005; Biasoto et al., 2010; Sáenz-Navajas et al., 2012). According to a number of studies astringency seems to be caused by the rupture of the lubricating saliva in the oral cavity rather than simple precipitation of salivary proteins (Sáenz-Navajas et al., 2012). In that sense it is explained how other wine constituents than flavanols, such as low molecular weight polyphenols phenolic acids and organic acids mainly described as bitter or sour, can enhance astringent sensation in wine (Sáenz-Navajas et al., 2012). Concerning possible interactions, a decrease in wine acidity (increase in pH) has a negative effect on perceived astringency. Moreover, an increase in fruity aromas of wine has the same effect due to cognitive link between sweetness and fruitiness (Sáenz-Navajas et al., 2012).

2.4. Sensory evaluation

The quantitative descriptive analysis (QDA®) is considered a primary measurement method for the sensory evaluation of wine aspects (Cozzolino et al., 2005; Vilanova et al., 2010). QDA® includes two steps; training on the products and on the method, as well as final profiling of products. The number of panellists included in a QDA® panel ranges from 10 to 12 persons and the QDA® training process lasts between 10-15 hours (Lawless & Heymann, 2010). During the training, panellists with the guidance of panel leader, generate the sensory vocabulary that will be used for the final profiling of the product. The training of QDA® panels requires the use of the specific product and ingredient references (Lawless & Heymann, 2010). The list of attributes to be included in the final profiling session and their meaning are discussed, throughout the training, among the panel and the panel leader (Næs et al., 2010). The discussion between the panel and the panel leader prior to the final profiling is used to calibrate and achieve consensus among the panel on the use of sensory words. In order to fulfil that purpose a few samples, usually the one’s that represent extreme states of intensity for certain attributes, are used to form the basis of the discussion (Næs et al., 2010) A 15 cm line scale, that was developed for this method, is used to evaluate and quantitate the intensity of each attribute in the final evaluation (Stone et al., 1974)

The QDA® has several advantages compared to other methods such as Flavour Profile® and Texture Profile. As the names of these methods reveal, Flavour Profile® is focussing on flavour, while Texture profile® only on texture (Lawless & Heymann, 2010). On the other hand in QDA® panellists can evaluate more aspects of the product simultaneously (Lawless & Heymann, 2010). Moreover in QDA® include generation of sensory words by the panel instead of use of standardized words and references like in the Sensory Spectrum® method (Lawless & Heymann, 2010).

2.5. Instrumental analysis

Instrumental methods are a fast way of identifying important compounds that are responsible for the organoleptic characteristics and the overall quality of wine. Volatile composition affects greatly the overall quality of wines; to measure this composition, headspace analysis followed by gas chromatograph coupled with mass spectrometry (GC–MS) is commonly used (Vilanova et al., 2013; Liu et al., 2014). Gas
Chromatography (GC) is a highly efficient technique separating 300 individual volatile compounds in a single run. Two main detectors of GC are used for the wine analysis, Flame Ionization Detection (FID) or mass-selective-detection (MS) (Ortega et al., 2001). While GC–MS provides an effective and precise tool for odorant separation and detection, the gas chromatography/olfactometry (GC-O/FID) technique is also commonly used to identify the odour active constituents of wine. Moreover Near Infrared (NIR) spectroscopy is frequently used to determine the gross composition of wine parameters and non-volatile constituents of wine (Cozzolino et al., 2003; Cozzolino et al., 2007).

2.5.1. Volatile compounds sampling methods-Dynamic Headspace Sampling (DHS)

Before injection into the GC column, volatile components must be released from the wine matrix into the headspace of the sample (Nielsen, 2010). The volatile compounds present in the samples must be isolated by the employment of gentle methods to avoid the generation of artefacts that could complicate further analysis (Belitz et al., 2009). Dynamic headspace sampling (DHS), which is also called purge and trap, is an extraction method where volatile constituents are constantly swept from the headspace of the sample, by a large volume of inert gas (N2 or He). The volatiles removed by the constant gas flow are then driven to an appropriate trap where they are stored until further analysis (Bazemore, 2011). This step allows the concentration of the volatiles which is an important action since it can lowers the detection limit of the constituents (Bazemore, 2011). As the majority of the volatile components are considered to be apolar and/or lipophilic, traps containing TENAX TA material are commonly used since they are considered appropriate for collection (Bazemore, 2011).

As discussed above one of the important advantages of DHS is that it provides a continuous flow of carrier gas. The introduction and departure of the carrier gas into the vessel (Bazemore, 2011) highly resembles the conditions created by the retro nasal breathing mechanisms during the consumption of the product. Thus the volatile compounds extracted from the sample will highly resemble the attained volatile constituents during real time consumption of the sample. As a result DHS is more appropriate choice of extraction method when one wants to come closer to the sensory aroma and flavour experience gained by the product.

Moreover when compared to other sampling methods, such as Solid Phase MicroExtraction (SPME) which is widely used in wines, DHS offers a larger capacity for compounds compared to the limited capacity of SPME. The limited capacity of SPME can lead to competitive absorption which can result to some compounds being excluded from the trap (Bazemore, 2011). As mentioned above, the whole process also provides concentration of aroma compounds found in lower concentrations. This can be an advantage since volatile aroma compounds are often present in very low concentrations (Bazemore, 2011).

2.5.2. Gas Chromatography (GC)

The most efficient way of releasing volatile compounds into the GC column is by rapid heating of the TENAX TA trap (Bazemore, 2011). Once transferred to the inside of the column, by inert gas flow, the volatile compounds will partition with the stationary phase according to their polarity. Elution time of a specific compound depends on the polarity; strong polarity results in larger partitioning with the stationary phase and therefore higher elution time. The differences in elution times are therefore responsible for the separation of the volatile compounds in the GC column (Harris, 2010). The GC detector identifies the signal given by a compound when it elutes from the column; the signal at each given time responds to the
accumulated signals of all compounds eluted at that time. This procedure continues throughout the run of the analysis resulting in a chromatogram in which the height and area of peaks that are present can be used to quantify the concentration of compound present in the sample. The quality of the resulting chromatogram depends on several factors including type of sample and separation efficiency. In case of not complete separation, peak broadening (coelution of compounds), which can complicate further analysis, can occur (Harris, 2010; Nielsen, 2010). Enhancement of separation efficiency or run time can be achieved by alteration of the conditions under which the chromatographic run takes place; variations on temperature programme, gas flow, choice of carrier gas, injection method (split/splitless), injection volume etc. are used for this purpose (Harris, 2010; Nielsen, 2010).

2.5.3. Gas Chromatography coupled to Mass spectrometry (GC-MS)
Following the elution of the GC column and detection, volatile compounds are directed to the ionization chamber of a mass spectrometer (MS). Once the compounds enter the ionization chamber a charge is placed on the molecules, through a process called ionization, which results to neutral compounds being transformed to positively charged molecular ions, M+• (Harris, 2010; Nielsen, 2010). Since molecular ions are very unstable they subsequently fragment. The resulting fragmentation pattern is unique for each compound and enables its identification (Harris, 2010). Moreover separation of the fragments according to their mass-to-charge ratio s (m/z) is achieved, by subjecting them to an electrostatic field inside the mass analyzer (Nielsen, 2010). The electrical field is created inside the quadrupole by four conducting rods (electromagnets), which pair wise alter their voltage. During this process ions with “wrong” m/z -values will be deflected by the electrical field while compounds with the chosen m/z -value will be able to reach the detector (Harris, 2010). When placed in scan mode the entire m/z -span is detected in less than a second, providing many mass spectra during the course of the sample run (Harris, 2010). Each mass spectrum will show the abundance of each m/z -values at a certain time during the run (Harris, 2010; Nielsen, 2010).

As mentioned above, the extensive fragmentation of the molecular ions, due to electron ionization, results in unique patterns in the mass spectra. The resulting mass spectra can be reproducible when the same voltage is applied while ionizing the molecules. This quality is used to create reproducible mass spectra fragmentation patterns, which are employed for the identification of compounds. As a result, each peak in GC-MS chromatograms is provided with a mass spectrum which enables the identification of volatile compounds in the sample. For identification software programmes (NIST) which compare the obtained mass spectra to registered spectra libraries are used. The program also provides a “Quality” of the fit which is a measure for how accurate the fit is; for at positive match a quality of minimum 80 % is required (Harris, 2010; Nielsen, 2010).

2.5.4. Gas Chromatografy- Olfactometry (GC-O)
Gas chromatography/olfactometry (GC-O) is commonly employed for the identification of the odour active constituents among the total pool of volatile compound that exist in a wine sample (Chisholm et al., 1995; Cacho et al., 2012). This hybrid technique combines the separating power of GC with the specific selectivity and sensitivity of the human nose (Mahattanatawee & Rouseff, 2011). The Concept of the GC-O apparatus is the following; the samples are introduced into a GC, where the procedure described in 2.5.2 takes place, while at the end of the of the GC capillary column an olfactometer which enables the assessor to sniff the carrier gas is attached. The carrier gas that elutes from the olfactometer is evaluated by the assessor who is then able to determine in the chromatogram the positions which correspond to odour active
components (Belitz et al., 2009). All developed olfactometers share a single feature; they all allow humidified air to be added to the effluent stream from the GC column. This provision is quite important since it allows the cooling of heated gases (temperatures can reach up to 250°C) and can therefore prevent dehydration of the assessors nasal passages during sniffing (Mahattanatawee & Rouseff, 2011).

Physical discomfort can become an obstacle since assessors need to stay alert and focused on aroma detection and description throughout the evaluation of the sample. For that purpose the comfort of the assessors is crucial (comfortable seating position) and the chromatographic conditions are adjusted in order to minimize the sensory evaluation time to approximately 30 minutes (Mahattanatawee & Rouseff, 2011).

The major challenge of the GC-O technique is the correct identification of the volatile constituents responsible for producing aroma activity. For that purpose a variety of methods, to confirm the identity of the odour active compound, are used; use of Standardized Retention Index (RI) values; Aroma description matching; MS identification and Use of authentic standards (Mahattanatawee & Rouseff, 2011).

The importance of this method lies in the fact that usually compounds found in in high concentrations have little or no aroma activity while most aroma active compounds exist as low concentration volatiles. Therefore Chromatograms obtained by other detection methods (FID or MS), which are mainly focused on aroma compounds which generate high responses, do not usually correspond to the perceived aroma profile of the sample. Moreover the human nose is a selective and highly sensitive detector which has a limit of detection far lower than most instrumental detectors for the majority of compounds. While GC-O can determine the odour active constituents of the product the reconstitution of the actual aroma is a complex procedure which includes interaction between aroma compounds as well as between aroma compounds and food matrix (Mahattanatawee & Rouseff, 2011).

### 2.5.5. Near-Infrared spectroscopy (NIR)

NIR spectroscopy records vibrations of molecular bonds (C-H, O-H and N-H) of chemical constituents in overtones (700-1800 nm) and combination tones (1800-2500 nm) of IR. This information can be transmitted through quartz based optical fibres enabling the formation of a characteristic spectrum which could be used as a fingerprint of the sample (Downey, 1994; Andrade et al., 2008).

The advantages of NIR compared to other spectroscopic and conventional techniques are, little or no sample preparation and fast, non-destructive, no use of chemicals or generation of chemical waste respectively. On the other hand NIR is not a stand-alone technology; reference samples must be used to ensure reliability calibration results and separate calibrations for each constituent or parameter are needed. Thus the information gained from NIR spectra can be only easily assessed by computer software that allows the development of complex mathematical relationships between the multivariate and co-liner spectral data and constituents/parameters determined by conventional techniques (Andrade et al., 2008).

NIR analysis has the advantage of simultaneously determining numerous constituents or parameters in samples and it has been used to determine the chemical composition of both grapes and wines in past research (Cozzolino et al., 2003; Cozzolino et al., 2007).
2.6. Data analysis

2.6.1. Quality control of sensory profile data
Checking the performance of the sensory panel is a crucial for determining panel reliability, improvement the panel through training and for better handling the final actual data. There are several methods to be used separately or in combination to identify different kinds of problems that can occur during sensory analysis. These methods are used to give performance feedback to the panel throughout the training as well as control the final quality of the sensory data. In case a certain assessor or attribute creates major alterations in the quality of data these results could be discarded before moving to further statistical analysis (Næs et al., 2010).

Firstly it should be noted that sensory data are meaningful when panellists are well calibrated and have achieved a consensus in the meaning of the sensory attributes used for the analysis (Næs et al., 2010). Still individual differences between assessors will occur, these differences should not be rejected since the final results may be subjected to bias and imprecise conclusions may be drawn from them. These individual differences include, the use of intensity scale by the assessors (small differences are expected, large differences can create a problem to further analysis); consensus or confusion on the definition of certain sensory words (a high degree of consensus between assessors is needed); panellists ability to reproduce themselves (small error variance needed) and assessors ability to detect differences between the products (Næs et al., 2010).

2.6.2. Multivariate analysis in Sensometrics and Chemometrics
Apart from classic calibration models achieved through univariate linear regression new statistical multivariate tools have been developed for handling sensory and chemical data. There are several advantages multivariate tools have over univariate one’s. Among them an important advantage is that multivariate models can be approached in both deductive (confirmation of causality) and inductive (exploration of possible relationships between variable and data sets) ways (Bro, 2003; Martens et al., 2007).

The basis of all multivariate methods is principal component analysis (PCA) (Martens et al., 2007). PCA is the general name for a technique which uses sophisticated underlying mathematical principles to transform a number of possibly correlated variables into a smaller number of variables, called principal components (PCs). This transformation is conducted in such a way so the first PC accounts for the largest possible variance while each succeeding PC has the highest variance possible (after the first PC-1) while it remaining uncorrelated with the preceding components. PCA is a very useful tool when analysing large data sets since the reduced dimension data allows easy identification of patterns patterns and outliers (Jolliffe, 2005).

Multivariate analysis is commonly employed for interpreting sensory and chemical wine data as well as for discovering relationships between sensory and instrumental data sets in wine (Cozzolino et al., 2005; Vilanova et al., 2010; Vilanova et al., 2012; Pérez-Magariño et al., 2013).
3. Materials and Methods

3.1. Experimental Design and wine samples

The study design included four grape varieties (Rondo, Regent, Cabernet Cortis and Bolero) as well as two grape production areas for each of the varieties in question. This resulted in the 8 wine samples used for this study (Table 1). All grapes used for the wine production were cultivated in 2011, in Denmark and the vinification process was performed by the same producer and under the same conditions. Sensory and Chemical analyses were performed in all 8 samples created by the design; the samples can be seen in Table 1.

Table 1: Red wine varieties; Cultivation areas of each variety and resulting samples

<table>
<thead>
<tr>
<th>Variety</th>
<th>Area</th>
<th>Røsnæs (Ro)</th>
<th>Pometet (Po)</th>
<th>Næstved (Na)</th>
<th>Modavi (Mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rondo</td>
<td>Rondo_Ro</td>
<td></td>
<td>-</td>
<td>-</td>
<td>Rondo_Mo</td>
</tr>
<tr>
<td>Regent</td>
<td>Regent_Ro</td>
<td>-</td>
<td>Regent_Na</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. Cortis</td>
<td>-</td>
<td>C.Cortis_Po</td>
<td>-</td>
<td>C. Cortis_Mo</td>
<td></td>
</tr>
<tr>
<td>Bolero</td>
<td>Bolero_Ro</td>
<td>-</td>
<td>Bolero_Na</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

3.1.1. Vinification process

For practical reasons and to minimise oxidation, the grapes were cold stored after harvest for one night as recommended for small scale vinification by Becker and Kerridge (1972). The vinification process, for all red wines that were included in this study, was performed in the cellar facility for enological research at 'Pometet' at KU-LIFE.

All wines were produced based on batches of 20-25 kg grapes. Microvinification was performed in small glass fermentation vessels of 5-10L. After destemming and crushing, the grapes were skinfermented in 10 L plastic buckets with lid for 4 days at 25°C while they were punched down 3 times per day. For fermentation, the yeast strain DV10, Saccharomyces Bayanus, Selektion Champenoise, Lalvin was added to the clear juice. The fermentation was performed at 19°C and nutrients (Vitamon Ultra, Erbslöh) were also added in the total amount of 3g/10 litres with 1/3 of the quantity being added with the dehydrated yeast. The rest 2/3 of the nutrient quantity was added 2 days later together with the sugar, if there was a need for sugar addition. Addition of sugar was performed only in the case of sugar grape content being below 80 Öechsle and if so the final total amount never exceeded 80 Öchsle or more than 50g/L. After pressing fermentation was finished at 19°C and followed by malolactic fermentation for 3-4 weeks (Enoferm Alpha, Lallemann, 100mg/10L). All wines were cold stabilized at 3-4°C for 3-4 weeks and racked twice. No filtering was performed and sulphite was added as metabisulphite. In all stages of the vinification process, CO2 was used during handling to protect the grapes and wines from oxidation.

3.2. Sensory evaluation

During all steps of the sensory evaluation of this study (pre-study, training and profiling sessions) the same neutralizers (sparkling water and crackers) were used to clear the mouth after tasting a sample. Moreover, panellists were free to drink tap water whenever they felt necessary and were given instructions to use the
spittoons, which were provided to them, to get rid of the wine quantity left in their mouth after they tasted it. Panellists were instructed to try not to swallow any wine while tasting the sample.

### 3.2.1. Pre-study

Four trained panellists participated in a 3 hour pre-study of the 8 wine samples used in this study. During the pre-study an initial list of sensory terms, which describe the wine samples and could facilitate the panel in their development of the sensory descriptors, was generated. Moreover the wine samples were compared and the one’s which exhibited the biggest sensory differences were put in pairs with the purpose of using them for 2 pair comparisons during the training sessions.

The appropriate serving amount and serving temperature for the wine samples were also determined during the pre-study. Regarding the serving amount, 20 ml was deemed a sufficient quantity for a panellist to complete the evaluation of one wine sample. Moreover the results of the pre-study revealed that the aroma quality of the wine depends a lot to the serving temperature, the temperature of the wine rises when a panellist is swirling the wine glass to release the aroma and that an average panellist takes 1-2 minutes to evaluate one wine sample. Taking into account the aforementioned information as well as the literature, which suggests that the appropriate serving temperature for red wines room temperature (Ross & Weller, 2008), it was decided that the serving temperature of the wine samples should be around 15-16°C. This temperature could allow the panellists to evaluate the aroma of the wine, by swirling the glass and smelling it, while the temperature is kept within the desirable range of 16-18°C.

### 3.2.2. Preparation for the sensory analysis

One hour before each sensory evaluation the wine bottles were taken out of the fridge (4°C) and were placed in water baths with ice. The ice was renewed throughout the procedure to keep the temperature of the water bath around 12°C. When the 20 ml quantity was placed in the wine glass it absorbed the temperature of the medium, reaching approximately 15-16°C which was the desirable serving temperature. The wine samples (20 ml) were served in ISO standard wine glasses (150 ml) with a three digit code and the temperature was measured right before serving, with an electronic thermometer, to confirm that it was inside the desirable range. The three digit codes used were generated randomly.

All the flavour and taste references were provided in 96 ml plastic SOLO cups with lids. Concerning the aroma references, they were placed in 96 ml plastic SOLO cups with the exception of references that were based on wine. Those were served in a wine glass at the appropriate temperature of approximately 15-16°C. There was a provided reference for all aroma and taste descriptors and for most of the flavour one’s. For some of the flavour descriptors the panel had to correlate the flavour with the matching smell. Similar to the pre-study sparkling water, tap water, crackers and spittoons, to discard the wine after the tasting, were provided for the panel.

### 3.2.3. Sensory vocabulary development, training and profiling

The sensory panellists were recruited from the University of Copenhagen, Faculty of Life Sciences, Denmark. The sensory panel consisted of 8 women and 3 men of various nationalities, with an age range of 23-26 years of age. The majority of the panellists had prior knowledge on sensory analysis. The sensory evaluation consisted of three training and one sensory profiling session. Each session was placed on a different day and lasted, approximately two hours. The training had two main objectives; to clarify, on a
cognitive level, the sensory terms used in the process and to train the panel how to use the scale to score the intensity of the attributes found in the samples. The initial vocabulary, which was developed during the pre-study, as well as a red wine aroma wheel, was given to the panel during the training sessions to help them develop the final profiling vocabulary. Moreover for each of the sensory terms used in the training process a reference sample, that matched the description of the odour, flavour, taste or sensation was provided. The final profiling vocabulary was determined during the training sessions by the panel with the collaboration of the panel leader. The final 24 sensory descriptors used in the profiling sessions, as well as their definitions, are given in Table 2. After the second and third training session, panel consistency was evaluated with the use Panel-Check version 1.2.1 (MATFORSK, Norway) and the results were used to give personal feedback to the panellists about their performance. Feedback has been shown to improve the panellist’s performance, panel’s consistency as well as reduce the training time of the panel (Ross & Weller, 2008). During the sensory training and profiling a 10 cm scale, with the anchors none and intense, was used to score the sensory terms. In the profiling day the panel received all three replicates of the full sample set (24 wine samples in total). The profiling day was separated into three sessions with ten minutes break in between. The samples were served with within block randomization, with each session representing one replica of the full sample set. The data was collected by Fizz Acquisition, Version 2.20C, 1994–2006 Biosystemes.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Descriptors</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>O_Red Berries</td>
<td>Red berries odour</td>
<td>Odour associated with strawberries and raspberries</td>
</tr>
<tr>
<td>O_Black Berries</td>
<td>Black berries odour</td>
<td>Odour associated with blueberries and blackberries</td>
</tr>
<tr>
<td>O_Citrus</td>
<td>Citrus odour</td>
<td>Odour associated with lemons and/or oranges</td>
</tr>
<tr>
<td>O_Green</td>
<td>Green odour</td>
<td>Fresh, leafy odour reminiscent of freshly cut grass</td>
</tr>
<tr>
<td>O_Floral</td>
<td>Floral odour</td>
<td>Jasmine and/or rose odour</td>
</tr>
<tr>
<td>O_Plums/Raisins</td>
<td>Odour of Plums and Raisins</td>
<td>Odour reminiscent of plums and/or raisins</td>
</tr>
<tr>
<td>O_Woody</td>
<td>Woody odour</td>
<td>Odour that reminds wet wood and/or pine</td>
</tr>
<tr>
<td>O_Tobacco</td>
<td>Tobacco odour</td>
<td>Tobacco and/or smoked odour</td>
</tr>
<tr>
<td>O_Black pepper</td>
<td>Black pepper aroma</td>
<td>Odour reminiscent of black pepper</td>
</tr>
<tr>
<td>O_Earthy</td>
<td>Earthy odour</td>
<td>Odour associated with wet soil</td>
</tr>
<tr>
<td>O_Chocolate</td>
<td>Chocolate odour</td>
<td>Odour associated with dark or milk chocolate</td>
</tr>
<tr>
<td>O_Animal/Leather</td>
<td>Animal/Leather odour</td>
<td>Off-odour associated with animal products (cat, rabbit pee etc.) which can have leather nuances</td>
</tr>
<tr>
<td>F_Red Berries</td>
<td>Red Berries flavour</td>
<td>Flavour associated with strawberries and raspberries</td>
</tr>
<tr>
<td>F_Plums/Raisins</td>
<td>Plums/Raisins flavour</td>
<td>Flavour reminiscent of plums and/or raisins</td>
</tr>
<tr>
<td>F_Citrus</td>
<td>Citrus flavour</td>
<td>Flavour associated with lemons and/or oranges</td>
</tr>
<tr>
<td>F_Green</td>
<td>Green flavour</td>
<td>Fresh, leafy flavour reminiscent of freshly cut grass</td>
</tr>
<tr>
<td>F_Spicy/Bl. Pepper</td>
<td>Spicy/Bl. Pepper flavour</td>
<td>Spicy flavour, reminiscent of black pepper</td>
</tr>
<tr>
<td>F_Smoked</td>
<td>Smoked flavour</td>
<td>Smoked flavour that can have tobacco nuances</td>
</tr>
<tr>
<td>F_Woody</td>
<td>Woody flavour</td>
<td>Flavour with woody notes</td>
</tr>
<tr>
<td>F_Animal/Leather</td>
<td>Animal/Leather flavour</td>
<td>Off-flavour associated with animal products (cat, rabbit pee etc.) which can have leather nuances</td>
</tr>
<tr>
<td>T_Sweet</td>
<td>Sweet taste</td>
<td>How sweet is the sample</td>
</tr>
<tr>
<td>T_Sour</td>
<td>Sour taste</td>
<td>How sour is the sample</td>
</tr>
<tr>
<td>T_Bitter</td>
<td>Bitter taste</td>
<td>How bitter is the sample</td>
</tr>
<tr>
<td>S_Astringent</td>
<td>Astringent sensation</td>
<td>Dryness in the mouth after sipping the sample</td>
</tr>
</tbody>
</table>

*These definitions were given on a paper to all the panellists during the training sessions.

### 3.3. Chemical analyses

Choices of methods for the chemical analysis of wine were based on the purpose of the study as well as the suitability of the methods to fulfil the purpose and the equipment available. In order to identify the aroma compounds in the wine samples GC-MS analysis was conducted. For GC-MS sample preparation dynamic
headspace sampling with TENAX traps was used. GC-O analysis was conducted to identify the odour active aroma compounds out of the total pool of the volatile compounds in the wine. Finally wine scanner analysis was performed to identify and quantitate non-volatile constituents as well as gross chemical composition of wine.

3.3.1. GC-MS analysis

Volatile compounds were collected on Tenax-TA traps. The traps contained 250 mg of Tenax-TA with mesh size 60/80 and a density of 0.37 g/mL (Buchem bv, Apeldoorn, The Netherlands). For each sample 20 ml of oil and 3 replicates were used. The samples were equilibrated to 37 ±1°C in a circulating water bath and then purged with nitrogen (100 mL/min) for 20 min. After the extraction of volatiles, 10 min purging with nitrogen was used to dispose any of water in traps that could create problems to further analysis. The trapped volatiles were desorbed using an automatic thermal desorption unit (ATD 400, Perkin Elmer, Norwalk, USA). Primary desorption was carried out by heating the trap to 250°C with a flow (60 mL/min) of carrier gas (H2) for 15.0 min. The stripped volatiles were trapped in a Tenax TA cold trap (30 mg held at 5°C), which was subsequently heated at 300°C for 4 min (secondary desorption, outlet split 1:10). This allowed for rapid transfer of volatiles to a gas chromatograph-mass spectrometer (GC-MS, 7890A GC-system interfaced with a 5975C VL MSD with Triple-Axis detector from Agilent Technologies, Palo Alto, California) through a heated (225°C) transfer line. Separation of volatiles was carried out on a DB-Wax capillary column 30 m long x 0.25 mm internal diameter, 0.50 µm film thickness. The column pressure was held constant at 2.4 psi resulting in an initial flow rate of approximately 1.2 mL/min using hydrogen as carrier gas. The column temperature programme was: 10 min at 30°C, from 40°C to 240°C at 8°C/min, and finally 5 min at 240°C. The mass spectrometer was operating in the electron ionisation mode at 70 eV. Mass-to-charge ratios between 15 and 300 were scanned. Volatile compounds were identified by probability based matching of their mass spectra with those of a commercial database (Wiley275.L, HP product no. G1035A).

3.3.2. GC-O analysis

Two expert judges were used for GC-O analysis of wine. In order to minimize the physical discomfort of the judges each sample run (52 minutes) was divided in two equal parts which were evaluated by different judges. The GC-O analysis of wine samples was performed in duplicates and each of the judges smelled one whole set of samples. The aroma quality and time of elution of odour active constituents was recorded during the GC-O evaluation.

GC-olfactometry was carried out using a Hewlett-Packard 5890 series II plus GC equipped with an SGE Olfactory Detector Outlet ODO-1 using the same type of column and the same GC-settings as for GC-MS, except that splitless injection was used. The effluent from the GC column was split, directing 20% of the flow to an FID detector and 80% of the flow to the Olfactory Detector Outlet. For desorption of the trapped volatiles, a Short Path Thermal Desorber model TD-4 was used.

3.3.3. Wine scanner analysis

The wine scanner analysis was performed with a WineScan FT 120 Fourier Transform Infrared Spectrophotometer (Foss, Hillerød, Denmark). Some samples with high CO2 concentrations needed a pre-treatment to lower the levels of CO2 in the sample before wine scanner analysis. High CO2 values can affect the wine scanner measurements of other variables. The wine scanner used calculated pH, malic acid, ethanol, lactic acid, Volatile acidity, glycerol, fructose, glucose, tartaric acid, density, CO2, Folin C. Index,
reducing sugar and total acid values of the wine samples. Wine scanner measurements were performed in duplicates.

3.4. Data analysis

3.4.1. Pre-processing of data
Panel-Check version 1.2.1 (MATFORSK, Norway) was used to visualise the initial sensory data and evaluate the performance of the descriptive panel. Firstly a visual inspection of the raw data was performed, this could help in the detection of extreme outliers or errors that should be removed before proceeding to further analysis (Næs et al., 2010).

Tucker-1 plots and p-MSE were used to check the general performance of the panel as well as the discriminative ability and repeatability of the assessors individually. Tucker1 correlation loadings plots showing the attributes for a specific assessor can reveal if the assessor discriminates well between the sensory descriptors; clustering of sensory words indicate similar interpretation. The 50% and 100% ellipses included in the plot are an indication of the explained variance of the variables; and for a well-trained and calibrated panel the variables should be located close to the outer ellipse (Næs et al., 2010). p-MSE plots were used since they illustrate the ability of an assessor to detect differences between products. p values that are plotted in the vertical axis while MSE values are plotted on horizontal axis of the same plot. In the plot p-values represent the assessor’s ability to discriminate between products, with low p indicating good discriminative ability, while repeatability of the assessors is given by the MSE value with low MSE indicating good repeatability. Therefore the best assessors are considered the ones who achieve both low p and low MSE values (Næs et al., 2010).

For identification and quantification of the initial GC-MS data the software program Chemstation was used. The peaks, that corresponded to the volatile compounds in the sample, were identified by probability based matching of their mass spectra with those of a commercial database (Wiley275.L, HP product no. G1035A). The peak areas were not transformed into absolute amounts, since no internal reference samples (pure standards) were not used for this analysis. Peak areas were used as values, instead of compound concentrations, in all proceeding data analysis. Peak areas can be used to, to compare the abundance of one compound between samples, but not compare the amounts of several compounds within one sample.

For acquired wine scanner data, variables with zero values (such as glucose) were excluded from further analysis. The CO2 variable was not taken into account due to effects the wine samples pre-treatment had on this value.

3.4.2. Sensory data
Data were subjected to univariate data analysis using SPSS statistics, version 22.0 (IBM). The model included Grape variety and Production area as fixed effects while sample replica and Judge were treated as random effects. Two way interactions were included if they were significant. Sensory descriptors were considered significantly different with a the significance level of 0.05 (p≤0.05).

All insignificant sensory descriptors were eliminated before performing multivariate data analysis. Sensory multivariate analysis was performed using Unscrambler X, version 10.2 (CAMO, ASA, Norway). A PCA biplot,
which included both the samples and the sensory descriptors in the matrix, was performed with averaged values over replica and judge. The biplot is closely related to principal component analysis (PCA). The idea behind it is to give a simultaneous display of observations (sensory/chemical values) and variables (samples) on the same two-dimensional space. The biplot provides illustrated information about the relationships in between the variables (samples) as well as between variables and observations (sensory/chemical values) (Jolliffe, 2005). The biplot gave an overview of the main sensory characteristics, the sensory differences as well as the sensory relationships between the wine samples.

### 3.4.3. GC-MS data

Firstly a Discriminate Partial Least Square Regression (D-PLSR) was carried out in order to reveal aroma compounds which contribute significantly ($p \leq 0.05$) to the perceived variation between the wine samples. Discriminant Partial Least Square Regression (D-PLSR) is used to discriminate between different classes of samples and use this to classify new samples. D-PLSR model was performed with Predictor matrix (X matrix) the volatile compounds and the response matrix (Y matrix) the 0/1 indicator variable matrix. Marten’s uncertainty test was used in order to identify the aroma compounds which contribute significantly ($p \leq 0.05$) in the in between wine sample differences (Martens & Martens, 2000). Marten’s uncertainty test uses a jack knife elimination of noisy variables (Martens & Martens, 2001).

Only significant sensory variables were forwarded to quantitative ANOVA Partial Least Square Regression (A-PLSR). A more stable and better explained model is achieved with eliminating the insignificant variables prior of performing the A-PLSR analysis.

The A-PLSR model was used to assess the effect of the design variables and illustrate which samples significantly differ from each other as well as determine the level and pattern of contribution of the samples to the variation in aroma data. The A-PLSR model was performed with the predictor matrix (X matrix) as 0/1 indicator variable matrix and the response matrix (Y matrix) the GC-MS data. Again jack Knife elimination was used in order to indicate which products (wine samples) significantly differ ($p \leq 0.05$) from each other (Martens & Martens, 2001).

Multivariate analyses (D-PLSR and A-PLSR) were run using individual values of samples given by replicate for the chemical data by the software programme Unscrambler X, version 10.2 (CAMO, ASA, Norway). All PLSR models were full-cross validated and data were auto-scaled prior to analysis.

### 3.4.4. GCO data

The odour active constituents of the wine samples were identified with the help of the Retention index (RI) values, aroma quality, as well as threshold values vs concentration od compounds. To achieve that purpose the same alkane standard solution was injected to both GC-O and GC-MS and the RIs of the compounds present in all samples were calculated. The RI given by the retention time of odour active compound in the GC-O was then matched to the compound with the same or similar RI in the GC-MS. The RIs between GC-MS and GC-O were comparable since the same column and settings are used in both machines. To confirm the match, the aroma quality of the compounds (obtained by literature research) was compared to the aroma description the judge recorded in the specific retention time during the sniffing of the sample. The fact that compounds with low sensory thresholds are more probable to be odiferous, especially when they
are found in high concentrations in the sample, was kept in mind while identifying the odour active constituents.

3.4.5. Wine scanner data
The wine scanner data followed the same data analysis with sensory data. The only difference existed in the pre-treatment of the data before the PCA bi plot analysis. Since there were no judges, the data was averaged only over replica.

3.4.6. Linking sensory to chemical data
In order to elucidate possible relationships between the sensory and chemical data a two block PLSR was performed with the significant chemical variables in the X matrix and the significant sensory descriptors in the Y matrix. Two-block PLSR is a modelling method which can be used to extract factors or latent variables which are linear combinations of one set of samples (such as instrumental data) that predict much of the variation in another set of samples of variables (such as sensory attribute ratings) (Martens et al., 2007). In that way it can be seen in what extend the chemical variables (X matrix) can be used to predict the sensory quality of the product (Y matrix). All the data in X matrix was auto-scaled prior to the analysis. Averaged values over panellists and sensory replicates were used for the sensory data and average values over replicates for the GC-MS and wine scanner data.

Moreover a second two block PLSR which included the odour active constituents instead of the significant GC-MS variables performed. The X matrix included all odour active volatiles and all significant wine scanner variables with the exception of folic C index. The Y matrix included all significant sensory descriptors with the exception of bitter taste and astringent sensation. Bitter taste and astringent sensation were excluded since the wine scanner produced no chemical variables they can be correlated with. On the other hand it has been proven that sweet taste can affect the perceived aroma and flavour of wine and sour taste can be correlated with several wine scanner variables. Therefore variables responsible for creating sweet and sour taste or interact with these specific tastes or general aroma of wine were kept in the model. Again data in X matrix were auto-scaled prior to the analysis and averaged values over panellists and sensory replicates were used for the sensory data while average values over replicates for the GC-MS and wine scanner data. This second model was created to evaluate whether the odour active constituents (X matrix) give a better prediction of the sensory quality of the product when compared to the whole set of the significant chemical variables.
4. Results

4.1. Sensory analysis

4.1.1. Panel performance

The Results of the p-MSE plots (Figure 1) show that most of the assessors, with the exception of judge 2 & 11, have rather low MSE values (X axis). Medium and high MSE values are observed on some of the attributes of Judge 2 & 11, respectively. P-values are rather spread across the Y axis for most of the assessors (Figure 1) with the exception of Judge 3, 8 & 10. Judge 3, 8 & 10 display low p-values, for the majority of the attributes.

![Figure 1: p-MSE plots by assessor. In the X axis the MSE values are represented while the Y axis represents the p values.](image)

The Tucker-1 overview correlation loadings plot of the attributes (Figure 2) show that for most of the assessors, with the exception of Judge 9 and 11, the majority of the attributes are located close or between the 50% and 100% ellipses.

More specifically comparing the individual correlation loadings plot of Judge 6, 9 and 11 it is revealed that for Judge 9 the majority of the attributes are spread out in the centre of the 50% ellipse while Judge 11 has only 6 attributes located between the 50% and 100% (Figure 3). Moreover a lot of groupings of attributes are formed in the centre of the 50% ellipse (Figure 3). For Judge 6 most of the attributes are spread out between the 50% and 100% ellipse (Figure 3).
Characterization of aroma and flavour profile of red cultivars from Denmark

4.1.1. Sensory profile of wines

The results of the univariate analysis indicated that 15, out of the 25, sensory descriptors which were used for the sensory analysis are useful (ps0.05) for discriminating between the samples. The descriptors that vary significantly for grape cultivation area and/or wine variety as well as their significance level can be seen in Table 3.

Table 3: The level of significance (ps0.05) of the sensory descriptors; the insignificant descriptors are not included in the table.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>Wine variety</th>
<th>Grape cultivation area</th>
<th>Wine variety*Grape cultivation area</th>
</tr>
</thead>
<tbody>
<tr>
<td>O_ Red Berries</td>
<td>-</td>
<td>0,009</td>
<td>-</td>
</tr>
<tr>
<td>O_Black Berries</td>
<td>-</td>
<td>0,009</td>
<td>-</td>
</tr>
<tr>
<td>O_Plums/Raisins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O_Tobacco</td>
<td>0,003</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O_Black pepper</td>
<td>0,000</td>
<td>0,024</td>
<td>0,001</td>
</tr>
<tr>
<td>O_Animal/Leather</td>
<td>0,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F_Plums/Raisins</td>
<td>-</td>
<td>0,000</td>
<td>-</td>
</tr>
<tr>
<td>F_Citrus</td>
<td>0,044</td>
<td>0,001</td>
<td>-</td>
</tr>
<tr>
<td>F_Spicy/Bl. Pepper</td>
<td>0,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F_Smoked</td>
<td>0,001</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F_Animal/Leather</td>
<td>0,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T_Sweet</td>
<td>-</td>
<td>0,009</td>
<td>-</td>
</tr>
<tr>
<td>T_Sour</td>
<td>0,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T_Bitter</td>
<td>0,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S_Astringent</td>
<td>0,000</td>
<td>0,004</td>
<td>0,032</td>
</tr>
</tbody>
</table>
Only the sensory descriptors that show a significant variation between the wine samples are used to make a Bi-plot. The explained variance of PC1 and PC2 is 79%, while the total explained variance of the three first PCs reaches 87%. The effect of wine variety and grape cultivation area on wine sensory quality is illustrated in the model.

Examining the correlation loadings plot of both Figure 4 and Figure 5 it can be seen that O_Black pepper is highly correlated with F_Black pepper and that both these descriptors are placed near the Regent Rosnaes sample which is located in the upper part of the plot. Regent Naestved sample is perceived to have a lower
Characterization of aroma and flavour profile of red cultivars from Denmark

Intensity of those attributes and is placed closer to the centre of the plot in Figure 4. Rondo Rosnaes and Modavi are highly correlated and placed in the centre of the plot away from any immediate sensory descriptors. Moreover O_Tobacco and F_smoked seem to be positively correlated with each other and located in a shared space between the Regent Rosnaes and the Cabernet Cortis samples. Concerning the Cabernet Cortis samples, they are both located in the right part of the plot, close to each other, and are surrounded by a high number of sensory descriptors. S_astringent is highly correlated to T_sour, both these descriptors as well as T_bitter seem to be highly perceived in the Cabernet Cortis samples. Additionally O_black berries, O_plums/raisins, F_plums raisins and O_Red berries are closely positioned to each other and to the Cabernet Cortis samples but are placed more closely to the centre of the plot when compared to S_astringent, T_sour and T_bitter. Concerning the Bolero samples, they are positioned next to each other and at the left part of the plot. T_sweet and O_animal/leather are placed closely to these samples, with O_animal/leather occupying a place closer to the outer part of the plot. Finally F_animal/leather and F_citrus are occupying the space between the Bolero and the Cabernet Cortis samples.

The correlation plot of PC2 vs PC3 (Figure 5) shows a related image with the difference that Cabernet Cortis samples are divided from each other and placed in opposite parts of the plot. This difference has as a result T_Bitter, T_Sour and S_astringent to be now placed near the middle of the plot along with the Regent Naestved and Rondo samples. Once more, O_Red berries and F_plums and raisins are highly correlated with each other and positioned closely in the plot to the Cabernet Cortis sample from the Pometet area. Moreover T_sweet is now closely located to the aforementioned group of samples and descriptors. Concerning O_ and F_animal/leather they seem to be highly correlated with each other and perceived in high intensities in the Bolero samples. This pair of highly correlated variables is anti-correlated with another highly correlated pair of variables O_ & F_Black pepper. This negative correlation is more evident in Figure 5 but can also be seen in Figure 4. Finally concerning O_Tobacco and Smoked, it is a highly correlated pair of variables which is now placed right below the Regent Rosnaes on the plot.

![Spiderplot illustrating the perceived intensities of the significant sensory descriptors in the wine samples. A scale of 10 cm was used for the sensory profiling.](image)

Moreover a numerical view on the sensory analysis results of the significant descriptors can be found on the spiderplot of Figure 6.
4.2. Chemical analyses

4.2.1. Aroma analysis-GCMS
According to the results of the GC-MS analysis on the isolated volatiles, 116 different compounds were identified in total in the eight different wine samples. Discriminate partial least square regression (D-PLSR) analysis was conducted in order to investigate which volatile compounds contribute in the in between variation of the wine samples. From the D-PLSR analysis it is revealed that 59 out of the 116 total compounds are significant (p≤0.05) for discrimination between the wine samples. The D-PLSR plot is displayed in (Figure 7).

![Chemical analysis results: D-PLSR correlation loadings plot of PC1 vs. PC2 illustrating the aroma compounds that are significant for discriminating between the wine samples. Significant volatile compounds (p≤0.05) are marked with a circle around the dot that corresponds to the volatile compound. The wine samples are indicated with a red colour. The inner and outer ellipses represent r² = 50% and 100%, respectively.](image)

A-PLSR analysis is performed by including only the significant volatiles (p≤0.05) for discriminating between the wine samples in the model. The generated correlation loadings plots are given in Figure 8 and Figure 9. PC-1 and PC-2 account for 48% of the variation in the aroma compounds while the total explained variance of the first three PCs reaches 64%.

The A-PLSR plot of PC-1 vs PC-2 (as well as the one from PC-2 vs PC-3) shows that samples originated from the area of Rosnaes (Regent_Ro, Rondo_Ro and Bolero_Ro) and Pometet are significantly different (p≤0.05) from the other wine samples Figure 8 and Figure 9.
Characterization of aroma and flavour profile of red cultivars from Denmark

Figure 8: A-PLSR correlations loadings plot of PC1 vs. PC2 illustrating the wine samples which significantly differ (p≤0.05) as well as the effect the grape variety and cultivation area have on the production of aroma compounds between the wine samples. Significant descriptors are marked with a circle around the dot that corresponds to the sample. The inner and outer ellipses represent $r^2=50\%$ and $100\%$ respectively.

Figure 9: A-PLSR correlations loadings plot of PC2 vs. PC3 illustrating the wine samples which significantly differ (p≤0.05) as well as the effect the grape variety and cultivation area have on the production of aroma compounds between the wine samples. Significant descriptors are marked with a circle around the dot that corresponds to the sample. The inner and outer ellipses represent $r^2=50\%$ and $100\%$ respectively.
Regent Ro wine sample is placed on the upper part of the PC1-PC2 A-PLSR correlation loadings plot (Figure 8) closely located to a group of compounds, which are strongly correlated with each other, including 5 terpenes and terpene derivatives (alpha muurolene, alpha amorphene, citronellol, alpha terpineol, and cadalin), 2 esters (1, 4-diethyl butanedioate, ethyl 9 decenoate), 1,6,6 trimethyl-1,2-dihydronaphthalene (TDN) and 2-pentanol. A second group of compounds, correlated with each other, including 2 alcohols (2-nonanol, (z)-2-hexenol) and alpha-calacorene, is also located near the Regent Rosnaes sample and the aforementioned group of compounds.

C.Cortis Modavi and C.Cortis Pometet are located close together in the A-PLSR PC1 vs PC2 plot (Figure 8). A large amount of esters (hexyl acetate, 2-phenylethyl acetate, 3-methylbutyl acetate, butyl acetate and 2-methylpropyl acetate) which are highly correlated with each other and with the compounds ethyl heptanoate, 2-ethoxy propane and 1-propanol are placed in the immediate space of the C. Cortis Pometet sample. Another group of compounds that show some correlation with each other and with the previous group of compounds are again a group of esters (ethyl decenoate, ethyl 2-phenylacetate, ethyl benzoate, ethyl (E)-4 hexenoate and ethyl dodecanoate) which are located in the space between the C. Cortis Pometet sample and the centre of the plot. Five aroma compound variables including a group of esters (methyl decenoate, methyl octanoate and 2-methyl propyl octanoate), 1,3 Benzothiazole and 1-octanol are showing negative correlations with the two aforementioned groups and are placed in the opposite to C. Cortis Pometet sample on the plot. Concerning the C. Cortis Modavi sample it is placed inside the 50% ellipse on the left upper part of the plot, close to this sample 3 alcohols (1-penten-3-ol, 4-methylpentan-1-ol and 2-methylpropan-1-ol), 2,4 hexadiene, ethyl octanoate and ethyl furan 2-carboxylate are also located. Moreover a group of compounds highly correlated with each other including 2 alcohols (2-heptanol and (z)-2-penten-1-ol), 2 esters (ethyl 3-phenyl propanoate and ethyl (z) hexenoate) and styrene, are located in the space between the 100% ellipse and the C. Cortis Modavi sample.

Concerning the remaining five samples they form two groups which show within as well as between group correlations. These groups are closely located to each other in the right lower part of the A-PLSR plot of PC1 vs. PC2 inside the 50% ellipse (Figure 8). The first group includes Rondo Modavi and Regent Naestved and is closely located to two aldehydes (ethyl nonanal and methyl butanal) and an unknown compound, which are positively correlated with each other. The second group of samples is composed by Bolero Rosnaes, Rondo Rosnaes and Bolero Naestved. This sample group is closely located to a group of compound highly correlated to each other, including two aldehydes (acetaldehyde and benzaldehyde) and one alcohol (butane-2, 3-diol). 2,3 pentanedione is also located near the 100% ellipse in the same area of these samples.

In the PC2 vs. PC3 A-PLSR correlation loadings (Figure 9) plot two major differences are detected. Firstly Bolero Naestved sample is now located in the centre of the plot. Moreover a large amount of volatile compounds, including alcohols (2-heptanol, 4-methylpentan-1-ol), esters (ethyl (Z)-3 hexenoate, ethyl 3-phenyl propanoate), 2,4 hexadiene, decanal, ethyl furan-2-carboxylate, styrene and sulfur dioxide are now located inside the 50% ellipse of the left upper part of the plot stripped of any immediate samples. Rondo Modavi and Rosnaes are correlated to each other and placed in the immediate space of another group of esters (ethyl benzoate, ethyl heptanoate, ethyl (E)-4 hexenoate and propyl acetate), butane-2,3-diol, 2-
methyl butanal and 1-propanol. Regent Naestved is located near to the Rondo samples on the outer 100% ellipse and closely to ethyl nonanoate, diethyl carbonate and an unknown compound.

Secondly the C. Cortis samples are now negatively correlated with each other. C.Cortis is closely located to a group of alcohols (2-methylpropanol, 1-octanol, (z)-2-penten-1-ol, and 1-penten-3-ol) which are correlated with each other. While C. Cortis Pometet is located closely to same group of esters and 2-ethoxy propane as described by the A-PLSR PC1 vs. PC2 as well as a pair of positively correlated compounds (2, 3-pentanediione and butane-2,3-dione). Bolero Rosnaes is now correlated to the C. Cortis Modavi sample and is placed closely to a group of esters (ethyl decenoate, ethyl dodecanoate, ethyl octanoate and methyl decenoate) located between the 50% and 100% ellipses. Furfural, 3-methylpentan-1-ol, ethyl 2-phenyl acetate and acetaldehyde are correlated to each other and occupy now the space between the Bolero Rosnaes sample and the centre of the plot.

Finally looking at the A-PLSR correlation loadings plot of PC2 vs. PC3 (Figure 9) the Regent Rosnaes sample is again closely located to the same group of compounds as in the PC1 vs. PC2 plot which are highly correlated with each other as well as with ethyl decenoate and alpha calacorene which is now more closely positioned to this sample.

4.2.2. Aroma analysis-GCO

The GC-O analysis of the 8 wine samples revealed 36 odour active constituents out of the total pool of 116 volatile components. The majority of the identified odour active constituents belong to the ester group while some other classes of volatile compounds such as alcohols, terpenes and aldehydes are also included. The odour active compounds, their aroma according to literature as well their identification method are included in Table 4

During the GC-O sensory evaluation of the aroma of the Rondo sample a peppery smell that lasted approximately 2 minutes and was not correlated to any compounds in found in the GC-MS chromatogram was recorded. This smell was identified several minutes after 2-phenylethanol (last compound identified) which has an intense smell of rose. The absent of any correlation is explained by the fact that after 2-phenylethanol, both in the GC-MS and GC-O chromatograms, few compounds are detected because the increased noise ratio of that area. The aroma quality as well as the area in which this compound was found matches the description of the sesqiterpene Rotundone which imparts a characteristic peppery aroma to varieties it is found.
Table 4: Odour active aroma compounds, their experimental retention index values (GCO), aroma quality according to literature and method of identification.

<table>
<thead>
<tr>
<th>Exp. R.I.</th>
<th>Identified odour active compound</th>
<th>Aroma quality</th>
<th>Method of Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>898</td>
<td>Ethyl acetate</td>
<td>pineapple fruity,</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>913</td>
<td>2-methyl butanone</td>
<td>Musty, chocolate, nutty, malty &amp; fermented</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>919</td>
<td>3-methyl butanone</td>
<td>Fruity, Almond-like, Toasted, Malty, Green</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>943</td>
<td>Diacetil</td>
<td>Sweet, creamy, buttery, caramel</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>976</td>
<td>Ethyl 2-methyl propanoate</td>
<td>Sweet, fruity, alcoholic, fusel rummy</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>991</td>
<td>Propyl acetate</td>
<td>solvent celery fruity fusel raspberry pear</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1002</td>
<td>2-methylpropyl acetate</td>
<td>Sweet, fruity, apple banana</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1025</td>
<td>Ethyl butanoate</td>
<td>Sweet, fruity, tutti frutti,</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1054</td>
<td>Pentane-2,3-dione</td>
<td>Buttery, nutty, toasted &amp; caramel</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1059</td>
<td>Ethyl 3-methylbutanoate</td>
<td>Sweet, fruity, pineapple, apple, green &amp; orange</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td></td>
<td>Butyl acetate</td>
<td>Sweet, fruity, apple &amp; banana</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1068</td>
<td>Hexanal</td>
<td>Green, fatty, leafy, vegetative, fruity, woody</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1091</td>
<td>3-methylbutyl acetate</td>
<td>Sweet, banana, fruity with a ripe nuance</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1136</td>
<td>1-Butanol</td>
<td>Fermented, fusel oil, sweet, balsam &amp; wine</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1143</td>
<td>methyl hexanoate</td>
<td>fruity green banana honey</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1155</td>
<td>3-methylbutan-1-ol</td>
<td>whiskey, malt, burnt, alcoholic, cognac, fruity, banana</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td></td>
<td>2-methylbutan-1-ol</td>
<td>wine, onion, roasted wine fruity</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1185</td>
<td>Ethyl hexanoate</td>
<td>sweet fruity pineapple waxy green banana</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1242</td>
<td>1-pentanol</td>
<td>Balsamic, fermented, bready, yeasty, winey</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1274</td>
<td>Hexyl acetate</td>
<td>fruity green apple banana sweet</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1306</td>
<td>3-methylpentan-1-ol</td>
<td>fermented, cognac, wine, cocoa, g</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1385</td>
<td>Methyl octanoate</td>
<td>Waxy, green, sweet, orange, vegetative &amp; herbal</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td></td>
<td>Nonanal</td>
<td>fat, citrus, green, waxy, rose, fresh &amp; orange peel</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1470</td>
<td>2-Furaldehyde</td>
<td>sweet woody almond fragrant baked bread</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1519</td>
<td>Benzaldehyde</td>
<td>strong sharp sweet bitter almond cherry</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1538</td>
<td>Linalool</td>
<td>Citrus, orange, floral, terpy, waxy &amp; rose</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1564</td>
<td>1-octanol</td>
<td>waxy green orange aldehydic rose mushroom</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1608</td>
<td>Gamma butyrolactone</td>
<td>Creamy, oily, fatty</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1661</td>
<td>3-methylbutanoic acid</td>
<td>Cheese, dairy, acidic, sour, stinky, fatty, fruity</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1733</td>
<td>TDN</td>
<td>Liquorice</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1804</td>
<td>methyl-2-hydrobenzoate</td>
<td>Sweet, root beer, aromatic, phenolic &amp; camphoraceous</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1823</td>
<td>2-phenylethyl acetate</td>
<td>Honey, rosy, yeasty cocoa &amp; balsamic</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1839</td>
<td>Beta-damascenone</td>
<td>Woody, sweet, fruity, earthy, green &amp; floral</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1858</td>
<td>Ethyl dodecanoate</td>
<td>Sweet, waxy, soapy, creamy, floral nuance</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1871</td>
<td>Hexyl butanoate</td>
<td>Green, fruity, vegetative</td>
<td>RI, Aroma quality</td>
</tr>
<tr>
<td>1957</td>
<td>2-phenylethanol</td>
<td>Sweet, floral, rose</td>
<td>RI, Aroma quality</td>
</tr>
</tbody>
</table>

1Experimental RI on DB-WAX capillary column (30 m length x 0.25 mm i.d., 0.5 mm film thickness, Agilent J&W, USA)
3Each compound was identified based on the following criteria: comparison of RI obtained by GC-MS with the RI obtained by GC-O; comparison of the aroma quality of a specific compound (obtained through literature) with the aroma quality recorded by the judges for the same compound.
4Possible to be either of both compounds responsible for the odour recorded by the sensory GCO evaluation of wine samples due to similar GC-MS RI (4 point differences) and similar odour quality
5Due to coelution phenomenon between the peaks it is possible that they both account for the odour perceived by the judge
61,1,6-trimethyl-1,2-dihydronaphthalene
4.2.3. Wine scanner analysis

The results of the wine scanner univariate analysis revealed that 10, out of the 13, descriptors show a significant variation (p≤0.05) between the wine samples. The descriptors that don’t show significant variation are glucose levels (all values were 0) and wine density. Moreover CO₂ was not included due to the pre-treatment of the wine samples, which could have affected the levels of this variable. All the descriptors varied significantly (p≤0.05) for both grape cultivation area and wine variety with the exception of Malic Acid which was found significant only for the variety variable. Furthermore by examining Table 5 it can be seen that malic acid values are approximately 0 in all samples, with exception of the C.Cortis one.

Table 5: Average concentrations and standard deviation values of the significant wine scanner variables; the insignificant descriptors are not included in the table.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>RegentRo</th>
<th>RegentNa</th>
<th>RondoMo</th>
<th>RondoRo</th>
<th>BoleroNa</th>
<th>BoleroRo</th>
<th>C.CortisMo</th>
<th>C.CortisPo</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3.86±(0)</td>
<td>4.15±(0)</td>
<td>3.93±(0)</td>
<td>3.72±(0)</td>
<td>3.66±(0)</td>
<td>3.61±(0)</td>
<td>3.76±(0)</td>
<td>4.28±(0)</td>
</tr>
<tr>
<td>Malic Acid</td>
<td>0.07±(0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2±(0)</td>
<td>0.25±(0.06)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>9.68±(0.01)</td>
<td>10.03±(0)</td>
<td>10.85±(0)</td>
<td>10.38±(0)</td>
<td>10.38±(0)</td>
<td>10.16±(0)</td>
<td>10.36±(0)</td>
<td>13.26±(0.01)</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>2.41(±0.03)</td>
<td>2.89(±0.02)</td>
<td>1.95(±0.01)</td>
<td>2.02(±0.06)</td>
<td>1.99(±0.01)</td>
<td>1.46(±0.02)</td>
<td>2.24(±0)</td>
<td>2.65±(0.03)</td>
</tr>
<tr>
<td>Volatile Acidity</td>
<td>0.29(±0)</td>
<td>0.56(±0.01)</td>
<td>0.46(±0)</td>
<td>0.35(±0.01)</td>
<td>0.36(±0)</td>
<td>0.27(±0)</td>
<td>0.28(±0.01)</td>
<td>0.41±(0)</td>
</tr>
<tr>
<td>Glycerol</td>
<td>6.16±(0.04)</td>
<td>7.63(±0.07)</td>
<td>7.23(±0.17)</td>
<td>6.68(±0)</td>
<td>6.08(±0)</td>
<td>6.33±(0.09)</td>
<td>6.78±(0.05)</td>
<td>8.52±(0.01)</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.1(±0.04)</td>
<td>1.44(±0.01)</td>
<td>1.25(±0.07)</td>
<td>0.85(±0.06)</td>
<td>0.97(±0.04)</td>
<td>0.82(±0.01)</td>
<td>0.97±(0.02)</td>
<td>1.34±(0)</td>
</tr>
<tr>
<td>Total Acid</td>
<td>2.95(±0)</td>
<td>3.07(±0.01)</td>
<td>2.88(±0)</td>
<td>3.37(±0.01)</td>
<td>3.12(±0.01)</td>
<td>3.07(±0)</td>
<td>3.43(±0.01)</td>
<td>2.64±(0)</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>1.55(±0.01)</td>
<td>1.42(±0.02)</td>
<td>1.7(±0.04)</td>
<td>2.05(±0.17)</td>
<td>1.37±(0.02)</td>
<td>2.33±(0.01)</td>
<td>2.8(±0.08)</td>
<td>1.33±(0.01)</td>
</tr>
<tr>
<td>Folin C. Index</td>
<td>54.02±(0.30)</td>
<td>81.66(±0.19)</td>
<td>68.23±(0.39)</td>
<td>67.54±(0.52)</td>
<td>48.82±(0)</td>
<td>44.23(±0.40)</td>
<td>95.54±(0.26)</td>
<td>94.31±(0.40)</td>
</tr>
<tr>
<td>Reducing Sugar</td>
<td>1.1(±0.03)</td>
<td>1.72(±0.04)</td>
<td>1.3(±0.08)</td>
<td>1.09(±0.03)</td>
<td>1.72(±0.08)</td>
<td>1.85±(0.08)</td>
<td>1.97(±0.06)</td>
<td>2.31±(0.08)</td>
</tr>
</tbody>
</table>

Measurement unit g/L * expressed in % Volume

Only the descriptors varied significantly between the wine samples are used to make a Bi-plot. The total explained variance of PC1 and PC2 is 56%.

Figure 10: Bi plot of PC1 vs. PC2 model illustrating the wine samples as well as the wine scanner descriptors that are significant for discrimination (p<0.05) between the cultivation area and wine variety. The inner and outer ellipses represent r²=50% and 100% respectively.
Examining the correlation loadings plot of PC1 vs PC2 (Figure 7) it is revealed that the majority of the wine scanner descriptors are located on the left part of the plot. Volatile acid and Fructose and closely placed to Regent Naestved sample while ethanol, Folin C Index and Malic acid are positively correlated with each other and located in the area of the Cabernet Cortis Pometet sample. PH, lactic acid and glycerol are positioned in the space between the Regent Na and Cabernet Cortis Pometet. Tartaric acid and total acid are the only descriptors allocated on the right part of the plot between Cabernet Cortis Modavi and Bolero Rosnaes and Rondo Ro. The samples Rondo Modavi, Regent Rosnae and Bolero Naestved are grouped together and positioned on the right upper part of the plot close to Rondo Rosnaes.

4.3. Linking sensory to chemical data

The explained variance plot of Figure 11 indicate that the first PLSR model, which included only the significant chemical (p≤0.05) variables for discriminated between the different wine samples could predict in some extend the sensory quality. To make the aforementioned model only the variables that are significant for both sensory and chemical data are included. On the other hand the second PLSR model which included the odour active constituents (instead of the significant volatiles) could not explain the aroma and flavour of the wine Figure 11.

Figure 11: Explained variance of PLSR models 1 & 2; PLSR model 1: significant chemical variables (GC-MS, wine scanner) in the X matrix, significant sensory descriptors in the Y matrix; PLSR model 2: odour active volatiles & significant wine scanner variables (with the exception of folic C index) in the X matrix, all significant sensory descriptors (with the exception of bitter taste & astringent sensation) in the Y matrix

Figure 12 presents the correlation loadings plot of PLSR 1 model. Volatiles are grouped according to the major impact they have on the flavour and odour of the wine. Therefore sensory descriptors and chemical variables that are placed near to each other in the PLSR plot are considered to be correlated.

The following relationships can be found in the PLSR 1 plot of the correlation of the sensory descriptors with the chemical one’s Figure 12; 5 terpenes (Citronellol, a-amorphene, a-muurolene, cadalin, a terpineol), TDN, ethyl 9-decenoate as well as 2-pentanol are projected close to black pepper odour and flavour; Glycerol as well as propyl lactate, 3-methylpentan-1-ol and 5 esters (ethyl octanoate, 2-methylpropyl acetate, butyl acetate, ethyl 3-phenylpropanoate, 2-phenylethyl acetate) seem to contribute to the plums/raisins flavour; 3-methyl cyclohexene, 3-pentanol, 1-propanol as well as styrene, ethyl heptanoate, ethyl (z)-3 hexenoate and 2-heptanol seem to explain tobacco odour; odour red berries seems to be correlated to 4-methyl pentanol and 2,4 hexadiene; smoked flavour is projected close to ethyl decenoate ethanol and (E)-2 Hexen-1-ol; Acetaldehyde and 2,3 pentadione are located close to citrus flavour; Odour animal/leather is projected close to tartaric acid and ethyl 2-phenylacetate; animal/leather flavour as well
Characterization of aroma and flavour profile of red cultivars from Denmark

as plums/raisins odour are projected close to butan-2,3-dione, ethyl benzoate and ethyl furan-carboxylate; Bitter and sour taste as well as astringent sensation are not placed close to any immediate descriptors with the exception of reducing sugar; sweet taste is not closely correlated with any immediate descriptors, a relative correlation can be found with fructose and as well as 4 esters (hexyl acetate, ethyl heptanoate, 3 methylbutyl acetate and ethyl hexenoate);

Figure 12: PLSR correlations loadings plot of PC1 vs. PC2 model illustrating the correlation between sensory and chemical variables. The significant chemical variables in the X matrix and the significant sensory descriptors in the Y matrix; Suffix to sensory terms indicates method of assessment by panellist O=odour, F=flavour, S=Sensation. The inner and outer ellipses represent r²=50% and 100% respectively.
5. Discussion

5.1. Sensory analysis

5.1.1. Panel performance

Evaluating the performance of the panel is an important step before proceeding to further statistical analysis. From the multitude of plots that can be used to achieve that purpose p-MSE plots as well as Tucker-1 plots were the most useful for drawing conclusions in this study.

Examining the p-MSE plots of (Figure 1) it can be seen that judge 2 & 11 show medium and high MSE values on some sensory attributes, respectively. Thus Judge 2 and especially Judge 11 cannot replicate themselves while scoring specific attributes (Næs et al., 2010). Moreover examining the p-values (vertical axis) of the same Judges it is revealed that judge 2 & 11 have p-values rather spread across the horizontal axis (Figure 1) and therefore this result indicates that this pair of judges also have problems in discriminating between products.

While this p-value pattern also applies to the majority of the rest of the panel (with the exception Judge 3, 8 & 10) it does not create a problem since their MSE values are quite low (Figure 1). The low MSE values and spread p-value pattern indicates that while the panel do not use the whole list of attributes to discriminate between products, still they can replicate themselves and show low p values for the majority of the significant attributes. Therefore their general performance is perceived as good. Concerning judge 3, 8 & 10, they display both low p and low MSE values and therefore are considered the most reliable assessors (Figure 1).

The results of Judge 2 and especially Judge 11 could indicate insufficient training and lack of experience for those panellists. Since the time span of training was limited to 6 hours for this study there were no time to help those panellists improve and catch up to the rest of the panel. Thus their low discriminative ability and repeatability could affect the reliability of the general sensory results. Therefore an exclusion of either or both judges could be considered as an option. Before moving to any premature conclusions, an examination of Tucker-1 correlation loadings plot is needed.

An examining of the overview correlation loadings plots of Figure 2 reveals that the panel, with the exception of Judge 9 and 11, is well-trained and calibrated since majority of the sensory variables are located close or between the 50% and 100% ellipses (Næs et al., 2010). Again Judge 11 is not included in the aforementioned group since only 6 of the total attributes are located between the 50% and 100% (Figure 3). Moreover Judge 11 correlation loading plot suggests similar interpretation of a lot of sensory words since a lot of groupings of attributes are formed inside the plot (Figure 3). This clustering of sensory attributes is more evident when comparing the individual correlation loadings plot of Judge 6, 9 and 11 which show that for both Judge 9 and 6 the majority of the attributes are spread out inside the plot (Figure 3).
The high MSE, spread p-values and confusion in the meaning of attributes indicated that Judge 11, when compared to the rest of the panel, needed more training to improve his performance that therefore his results were excluded before moving to further analysis.

5.1.2. Sensory profile of wines

In order to investigate the relationships between the sensory variables as well as the effect of the grape variety and cultivation area on the sensory quality of the resulting wine, a PCA biplot including only the significant sensory descriptors (Table 3) was performed. While the explained variance of the first two PCs of the biplot of Figure 4 is quite high (79%) the third PC is also included (Figure 5) in the explanation of results. This is due to the high weight the C. Cortis samples have in the model. As a result the majority of the attributes are drawn towards this set of samples leaving the most of the other samples stripped of any immediate descriptors. What the PC-3 offers is that it divides the C. Cortis samples by negatively correlating them in the plot (Figure 5) and therefore reveals not only secondary sensory relationships between descriptors and other samples but also the effect of the production area on the C. Cortis sensory quality.

The Regent Rosnaes sample seems to be primarily characterized by black pepper odour and flavour (Figure 4; Figure 5). This is furthermore confirmed by the spiderplot of Figure 6 where it can be seen that the Regent Rosnaes sample is highly scored on those descriptors exceeding the values of other samples by 2-3 points in the odour. The odour and flavour of black pepper seems to originate majorly from the grape variety and to be enhanced by grape cultivation area. This could be deducted since this flavour and aroma also exists in the Regent Naestved sample in a smaller extend. This is evident when examining in both Figure 4 and. Figure 6 where it can be seen that the Regent Naestved sample has the second biggest values on these attributes. It is possible the micro climate of the Rosnaes area enhances the production of the constituents responsible for the black pepper characteristic aroma of this variety. Moreover the Regent Rosnaes variety seems to be perceived as having a smoked flavour and a tobacco odour (Figure 5). Smoked flavour and a tobacco odour seem to be minor sensory qualities since they achieve low scores (Figure 6) thus perceived in a much lesser extend when compared to the black pepper flavour and aroma of this variety. The Regent Naestved seems perceived as a much less intense version of the Rosnaes sample, since the same pattern of scoring is followed among the majority of the significant sensory attributes with the exception of astringent sensation (Figure 6).

The Cabernet Cortis samples are perceived to have similar sensory qualities and are being perceived as having a more black berries, red berries, plums/raisins and tobacco odour as well as more smoked, plums/raisins, leather/animal, bitter, sour and astringent taste and flavour when compared to other samples. Both Cabernet Cortis samples seem to be perceived equally bitter, sour and astringent since when those samples are anti-correlated in PC2 vs PC3 plot, these descriptors move to the centre of the Figure 5 plot. This can be also seen in the spiderplot of Figure 6 which reveals that not only both C. Cortis samples share those characteristics but that bitter, sour and astringent are major characteristics of this variety which achieve high score values. In the correlation loadings plot of Figure 5, where the C. cortis samples are divided, the effect of production area on the aroma and flavour characteristics of this variety is more evident. The Pometet area seems to play an important role since production on that area enhances the black and red berries odour as well as plums/raisins flavour considerably for the C.Cortis variety. Sweet taste is also perceived more highly in the C.Cortis Pometet sample but in general taste sweet scores low values in all samples, including C.Cortis Pometet, so the observed variation is quite small (Figure 6).
Moreover an off-flavour associated with animal/leather can be found in these wines (Figure 4) but the actual scoring values are again, as for sweet, rather low, so they do not characterize the aroma and flavour of the variety and could be considered as a nuance.

Concerning the Bolero samples, they are perceived as being quite similar and are described primarily by an animal/leather off odour and a sweet taste when compared to the other samples (Figure 4). Concerning the sweet taste, as mentioned before, it does not characterize the sample due to the general low scoring on this attribute. On the other hand the animal/leather off odour is quite intense on the Bolero Rosnaes sample, while the Bolero Naestved is again a less intense sample when compared to this sensory descriptor (Figure 6). Concerning the perceived animal/leather off-flavour it seems to be highly correlated with the presence of the corresponding aroma (Figure 5) but the recorded intensities and variations for this flavour are again quite low among the samples (Figure 6).

Finally the Rondo samples are perceived as being quite similar to each other (Figure 4). This is verified by the spiderplot of Figure 6 which reveals that both samples score medium intensities and show smaller variations between areas of production for the majority of the attributes included in this study (Figure 6). Moreover no specific sensory descriptors seem to uniquely characterize those samples. This makes Rondo a more balanced variety that seems to be less affected by the micro climate of the production area. This could be used as an advantage since the resulting wine has balanced flavour and aroma qualities that could be manipulated in blends by the introduction of wine varieties with more notable characteristics.

As it can be seen from the spiderplot (Figure 6) for the majority of the descriptors (with the exception of astringency) the panel uses only the lower 2/3 of the scale. This can be a result of the limited training time the panel had. With more training it is possible that the panel could use the whole 10 cm scale to evaluate the attributes giving more clear results on the differences between the products.

5.2. Chemical analyses

5.2.1. Aroma analysis-GCMS & GCO

Only the volatile compounds that contribute significantly (p≤0.05) in the in between variation of the wine samples are included in the A-PLSR correlation loadings plot of Figure 8 and Figure 9. All three PCs (total explained variance 64%) are kept since, similarly to the sensory results, the high weight the C. Cortis samples in the model leaves other samples less explained. Therefore the third A-PLSR dimension is used to reveal the relationships between volatile components and other samples by reducing the effect of C. Cortis samples by correlating them inversely.

According to the A-PLSR plot (Figure 8 and Figure 9) samples originated from the area of Rosnaes and Pometet are significantly different (p≤0.05) from the other wine samples. This agrees with the results of the sensory analysis which revealed that specific sensory characteristics are enhanced in those production areas. It seems that the conditions in these areas enhance the production specific volatile compounds which in their turn enhance the corresponding aroma and flavour of the resulting wine.

The compounds that are found in higher concentrations in the Regent Rosnaes sample are 7 terpenes and terpene derivatives, 2 alcohols, 2 esters (ethyl -9 decenoate and 1, 4-diethyl butanedioate) and TDN (1, 1,
6-trimethyl-1, 2-dihydronaphthalene). From these compounds only TDN seems to be odiferous according to the results of the GCO analysis, which can be seen in Table 4. The specific aroma and flavour of the Regent Rosnaes sample seems to be highly correlated to the existence of terpenes and TDN and can originate from the Regent cultivar, since according to literature these compounds derive from grapes (Bakker & Clarke, 2011; Styger et al., 2011). While according to the GCO results (Table 4) the terpenes compounds abundant in this sample are not odour active, the effect they have on wine aroma is synergistic and therefore they can affect the aroma flavour of the Regent Rosnaes even if their concentrations are below the threshold limit (Styger et al., 2011). Terpenes and TDN, which belongs to C13-norisoprenoids, contribute floral and kerose qualities to the wine, respectively (Ebeler, 2001; Bakker & Clarke, 2011). It seems that in addition to grape variety, cultivation area affect the concentration of terpenes and TDN, since contrary to the Rosnaes sample, the Regent Naested sample exhibits lower concentrations of these compounds (Figure 8 and Figure 9). The lower abundance in the Naestved sample could suggest that these compounds are found in their non-volatile glycosylated form instead of their free volatile one (Bakker & Clarke, 2011; Styger et al., 2011). Since the vinification process followed for the production of these wines is the same, lower content of the non-volatile form suggests more mature berries at harvesting in the Naestved area, when compared to Rosnaes one (Bakker & Clarke, 2011; Styger et al., 2011). Therefore lower ripening stage of the berries enhances the aroma of this variety. This has been suggested before by Ribéreau-Gayon et al. (2006) who explains that while hot climate enhances the sugar content of the grape it is not always favourable for wine quality. With respect to the terpenes family of compounds, which seems to affect significantly the flavour and aroma of the Regent Rosnaes sample, it has also been found to contribute to varietal wine aroma of other cultivars such as Muscat, Riesling and Shiraz (Bakker & Clarke, 2011; Styger et al., 2011). The intense black pepper aroma and flavour which characterizes this variety from a sensory perspective (Figure 4; Figure 6) as well as the high concentrations of terpenes suggest the presence of the sesquiterpene Rotundone. Rotundone can act as an impact compound giving a distinctive white/black peppery aroma to wine varieties such as Shiraz (Styger et al., 2011). Still no relevant data was found in the GC-MS results.

C.Cortis Modavi and C.Cortis Pometet seem to express more similar characteristics since they are located close together in in the plot of Figure 8. This suggests that the area of production has a smaller effect on the volatile content and therefore, in great extent, on the aroma and flavour quality of the wine, when compared to the Regent variety. This agrees with the results of the sensory analysis which indicates that the C.Cortis samples share several similar aroma and flavour qualities (Figure 4). Still only the Pometet sample is found to significantly differ from a volatile perspective (Figure 8 and Figure 9). A great number of esters are abundant in the Pometet sample (Figure 8). Esters mainly derive from fermentation and are major constituents when referring to wine aroma and flavour (Ebeler, 2001; Bakker & Clarke, 2011; Styger et al., 2011). As a general group, esters contribute fruity aromas in wine (Bakker & Clarke, 2011). However differentiations do exist within the group in the quality of the aroma they offer to wine; lower aliphatic ethyl esters give fruity characteristics whereas the higher esters release more soapy, oily and candle wax notes (Bakker & Clarke, 2011). Moreover the majority of the esters abundant in this sample have also been found to be odour active according to the GCO results (Table 4). The high amount of esters as well as the variability in the aroma quality they release explains in some extent the amount of fruity descriptors which are used to describe this sample from a sensory perspective. Therefore esters seem to have contributed in a great extend to the several aroma and flavour qualities which are found in the C.Cortis Pometet sample (Figure 4). C.Cortis Modavi, as well as some of its compounds, is located close to C.Cortis Pometet which is
abundant in alcohols and some esters. Alcohols are derived from the fermentation process, as a group when they are found in high concentrations they can have a negative impact to the sensory quality of wine (Ebeler, 2001). However none of the alcohol compounds which are found in C.Cortis Modavi are odour active according to the GCO analysis (Table 4). This is expected since according to literature the majority of the alcohols are usually found in concentrations below their detection threshold (Ebeler, 2001). Therefore the perceived sensory quality of C. Cortis Modavi can be mainly attributed to the ester compounds which are abundant in this sample, as well as possible synergistic effects between other constituents. While esters and alcohols are not considered varietal impact compounds, when discussing aroma and flavour of wine different ratios in compounds can also create a cultivars distinctive sensory quality (Styger et al., 2011). Therefore the specific aroma and flavour characteristics of the Cabernet Cortis variety can be attributed in some extend to high ester production during fermentation.

PC 1 vs PC 2 correlation loadings plot of Figure 8 reveals little for the compound composition of the remaining samples Regent Naestved, Rondo Modavi, Rondo Rosnaes, Bolero Rosnaes and Bolero Naestved. According to Figure 8 the main compounds which are abundant for this group of samples are a number of aldehydes (ethyl nonanal and methyl butanal, acetaldehyde and benzoaldehyde), 1 alcohol (butane-2,3 diol) and pentane-2,3-dione. The GCO results reveal that from the aforementioned group of compounds only benzaldehyde and pentane-2,3-dione, which confer sweet, bitter, almond, cherry and buttery, nutty, toasted, caramel aromas, respectively are odour active (Table 4). The reason this samples are stripped from any other volatile compounds is due to the high weight the C. Cortis and Regent Rosnaes samples. The lower weight of these five samples can be translated to lower concentrations, for the majority of compounds, when compared to C. Cortis samples and Regent Rosnaes. Still this doesn’t correlate to less distinctive wine aroma profile for these varieties since volatile compounds could be also present in high amounts in these samples.

In order to investigate this, correlation loadings plot of PC2 vs PC3 where the effect of Cabernet cortis samples is reduced can be used (Figure 9). According to the plot of Figure 9 Rondo Modavi and Rosnaes share similar volatile profiles, 4 esters (ethyl benzoate, ethyl heptanoate, ethyl (E)-4 hexenoate and propyl acetate), 2 alcohols (1-propanol, butane-2,3-diol) and 2-methylbutanal are abundant in both Rondo samples. According to the GCO evaluation the majority of these compounds are odour active (Table 4). Moreover the similar profile of the Rondo variety in both areas reveals that the sensory quality of the grape is less depended on area. This agrees with the sensory evaluation findings which suggest that the Rondo samples have similar sensory qualities independent of the production area (Figure 4;Figure 6). Concerning the suitability of this cultivar for wine production in Denmark this can prove an advantage. Denmark shows a great variability in the microclimate of the cultivation areas, therefore if the purpose is to produce wine with a steady sensory quality the Rondo variety seems to be the most appropriate (Bentzen & Smith, 2009).

Concerning the Bolero samples, in A-PLSR PC2 vs. PC3 correlation loadings (Figure 9) the Naestved sample is located in the centre of the plot while for the Bolero Rosnaes is now revealed that it shares some similar characteristics with the C. Cortis Modavi sample composition. The differences between the samples could once more be attributed on the effect the production area has on the quality of grapes. Grape variety and «terroir» define in a great extend the primary pool of compounds which will be transformed during vinification to create the final composition of wine. With respect to the Rosnaes sample, it seems to have
higher concentration of esters (ethyl decenoate, ethyl dodecanoate, ethyl octanoate and methyl decenoate,) 3-methylpentan-1-ol and acetaldehyde, when compared to the rest samples. From the aforementioned compounds only 3-methylpentan-1-ol was found above the threshold value in the sensory GCO evaluation of the samples (Table 4). 3-methylpentan-1-ol is a compound which confers a fermented, cognac, wine aroma to wine (Table 4). Moreover acetaldehyde, which is present in this sample, can be considered a fault in wine when found in high concentrations since this in high amounts of this compound release a pungent irritating odour reminiscent of green grass or apples (Styger et al., 2011). The off-flavour and odour reminiscent of animal/leather this cultivar exhibits (Figure 4; Figure 5; Figure 6,) which is more pronounced for the Rosnaes samples, may be partly attributed to acetaldehyde concentration and the fermented aroma of 3-methylpentan-1-ol or it can originate from other compound interactions.

5.2.2. Wine scanner analysis
While examining the results of the wine scanner, it is important to mention the significance in variations of the wine scanner variables may be virtual. The two replicas used for the wine scanner analysis are taken from the same sample amount. Therefore the results reveal more about the machine’s repeatability and should be used only as an indication of differences in concentrations between samples. The low standard deviations (std) this procedure creates (Table 5) often result to minor variations in concentrations to be rendered significant, a case which applies to malic acid. Malic acid concentrations are approximately zero for all samples with the exception of C.Cortis variety which has 0,20-0,25 value, which is a minor variation the amount of this acid. Still, due to the low std this variable is considered significantly higher for the C. Cortis cultivar. Malic acid induces a harsh, green taste to wine which is gradually lost during MLF, where it is transformed into lactic acid, which has a mild flavour (Biasoto et al., 2010; Bakker & Clarke, 2011). The zero levels of malic acid in the samples reveal that the samples that the MLF process was completed during vinification. While the small variation in the C. Cortis sample only reveals a tendency for this variety to accumulate higher levels of malic acid, part of which can remain after fermentation inducing a harsh taste to the wine. Therefore while examining the patterns found in the correlation loadings plot of PC1 vs PC2 (Figure 10), the actual concentration of wine scanner variables found in Table 5 should be always considered.

Moreover with respect to the C. Cortis, the correlation loadings plot of Figure 10 indicates that ethanol is found in higher concentrations in this sample. Indeed when examining Table 5 it can be seen that the Cabernet Cortis Pometet sample has 3% more ethanol content when compared to the rest of the samples which have an average 10% concentration. This suggests more sugar accumulation in this area, since alcohol is a direct metabolite of sugar transformation during fermentation, and therefore more optimal conditions during grape maturation (Ebeler, 2001; Bakker & Clarke, 2011). This is also suggested by the fructose results (Table 5) which reveal that C. Cortis Pometet has the second highest concentration of this compound. Unfortunately, more conclusive results cannot be drawn about the effect the Pometet area has on the maturation of the grapes since no other sample from this production site was included in the study. Concerning the effects of this compound on the aroma and flavour of wine, while ethanol has a low OAV when found in high concentration it can greatly influence wine quality. Furthermore it affects the sensory quality by interacting with other families of compounds which affect wine’s perceived aroma and flavour (Ebeler, 2001; Styger et al., 2011). It has been shown by Grosch (2001) that the reduction of ethanol by 2% induced an increased perception of fruity, flowery and acid flavours and aromas of the wine. Thus it should be expected that the 3% higher ethanol content of the Pometet sample, when compared to the Modavi
one should have the reverse effect by masking those aromas. This doesn’t agree with the sensory results since the Pometet sample is perceived as having a more intense sensory aroma and flavour profile when compared to the Modavi (Figure 6). It is possible that the compounds responsible for the aforementioned aromas are more abundant in the Pometet wine sample to begin with. Moreover, interactions between constituents could take place. On the other hand, increase in ethanol’s levels enhances the perceived astringency as well as bitterness resulting wine (Ebeler, 2001; Styger et al., 2011). This agrees with the sensory results that reveal that the Cabernet Cortis Pometet sample is perceived as the most astringent and bitter among all samples. Still the Cabernet Cortis Modavi has the same characteristics, which leads to the conclusion that these qualities are mostly affected by other constituents and not ethanol.

Volatile acid, which represents the acetic acid concentration, and fructose are more abundant in the Regent Naestved sample (Figure 10). However when looking at Table 5 the variation as well as the concentration of fructose among samples is quite small. Fructose and glucose, which has zero concentrations in all samples, are the main reducing sugars during fermentation and primary constituents of the sweet taste in wines. Due to Denmark’s cool climate conditions accumulation of sugar is an issue, therefore low levels of fructose are expected since the limited amount of reducing sugars found in the grapes are transformed during fermentation. This agrees with the sensory results that revealed that the level of perceived sweetness was similarly low for all samples (Figure 6). Still an increased concentration of fructose is expected in the Regent Naestved sample, since as discussed in the previous section, the Regent berries of this area seems to have achieved a higher level of maturation at harvesting compared to Rosnaes one. The same applies for the Bolero sample from the Naestved area which exhibits a higher amount of fructose when compared to Rosnaes samples of the same variety Table 5. This reveals a pattern which furthermore confirms that the climate conditions in the Naestved area enhanced the ripening process of the berries. With respect to acetic acid, it is a volatile organic acid which can have an undesirable effect in wines when found at high concentrations by attaching a Vinegar-like odour/flavour (Bakker & Clarke, 2011; Sáenz-Navajas et al., 2012). Acetic acid’s high concentration along with the lower expression of free forms of terpenes in the Naestved sample, when compared to the Rosnaes one could have partly masked the distinctive black pepper aroma and flavour of this cultivar (Figure 4; Figure 8).

Regent Na and Cabernet Cortis Pometet seem to have the higher content of lactic acid and glycerol as well as the highest PH values Figure 10. Glycerol is a compound which has a sweet taste but cannot affect the perceived sweetness by itself due to its high sensory threshold (Bakker & Clarke, 2011). With respect to the effect glycerol has on the general sweetness in wines, it has been shown that in some cases, depending on concentration and grape variety, it enhances the effect of perceived sweetness in wines (Styger et al., 2011; Sáenz-Navajas et al., 2012). Still a study by Nieuwoudt et al. (2002) revealed that the effect of glycerol concentration has not a significant effect on the quality of red wine. This finding agrees with the sensory findings that show small variation in the perceived sweetness among samples (Figure 6). Lactic acid, the product of transformation of malic acid during MLF, has a mild taste and it shows small variations between the samples Figure 5. Still, a consistent lower content of malic acid in the samples coming from the Rosnaes area, when comparing the two areas within a cultivar, suggests that the accumulation of the precursor compound, malic acid, is lower in this production site Figure 5. Since malic acid contributes to a harsh unpleasant taste, its lower accumulation is the Rosnaes area is positive aspect for this production site.
Tartaric acid and total acid are more abundant in the C. Cortis Modavi and Rondo Rosnaes samples Table 5. The reason Bolero Rosnaes is positioned between those two samples can be attributed to the small variations in the actual concentrations as well as the effect other variables have in the position of this sample Figure 10. Perceived sourness is enhanced with increased levels of total acid, which agrees with our results in the case of C. Cortis Modavi (Figure 6), while no such sensory data were found for the Rondo Rosnaes sample (Sáenz-Navajas et al., 2012). As a general rule, insufficient concentration of organic acids results to flat tasting wines, while excess concentration can induce an unpleasant acidity, suppress other desirable flavours and negatively affect the general quality of wine. (Biasoto et al., 2010; Bakker & Clarke, 2011; Sáenz-Navajas et al., 2012). In this study the level of acidity seems to contribute, along with other factors, to the enhancement the aroma and flavour characteristics of the varieties in question (Figure 6). With respect to tartaric acid, it can induce a sharp unpleasant taste to wine especially when found in concentrations above 5 g L$^{-1}$, still the initial concentration of tartaric acid was much lower than the threshold value for all samples (Biasoto et al., 2010; Sáenz-Navajas et al., 2012). Therefore tartaric acid levels do not seem to contribute negatively in the quality of wines included in this study.

Rondo Modavi, Regent Rosnaes and Bolero Naestved are grouped together away from any immediate wine scanner variables which suggest lower contents the wine constituents included in the wine scanner analysis when compared to the rest of the samples (Figure 10). This agrees with the results found in Table 5.

### 5.3. Linking sensory to chemical data

Flavour and aroma of wine are a result of a multitude of interactions between the volatile and non-volatile constituents of wines. The non-volatile matrix affects the release of volatile compounds while on the other hand the sensory characteristics attributed to non-volatiles are not fully realised in the absence of volatile constituents (Sáenz-Navajas et al., 2012). Therefore correlation between the chemical composition of wines and the resulting wine sensory quality is an interesting subject which can help the further development of wine industry as well as improve the aroma and flavour potential of several cultivars.

Two models were created in an effort to predict sensory quality from chemical composition of wine samples. While the first PLSR model that included only the significant chemical (ps0.05) succeeded in some extend to predict the significant sensory descriptors, the second PLSR model which included the odour active constituents could not explain the sensory attributes included in the model Figure 11. The main difference between these two models is that in the second model odour active constituents replaced the significant volatiles. Moreover, sensory descriptors (Bitter taste, astringent sensation) were excluded due to fact that they were no chemical variables for them to be correlated with. Astringency is a dry, puckering mouthfeel caused by wine consumption, tannins and more specifically procyanidins seem to be the main compounds contributing to this sensation in wines (Monagas et al., 2005; Biasoto et al., 2010; Sáenz-Navajas et al., 2012). Moreover bitter sensation of wine is mainly related to phenolic compound with low molecular weights (myricetin, quercetin and catechin) (Biasoto et al., 2010; Sáenz-Navajas et al., 2012). The wine scanner that was used for this project could only measure the total polyphenolic content (Folin C. Index) and did give concentrations for the individual groups of compounds, thus the exclusion of these variables was decided.
There are several reasons that can explain why one model failed while the other succeeded. Firstly the amount of odour active compounds is smaller compared to the amount of significant volatile constituents; therefore the model has less chemical variables to predict the sensory ones. Secondly when including only the odour active compounds, synergistic effects of constituents that are below their sensory thresholds and could affect greatly the wine quality (such as terpenes) are excluded (Styger et al., 2011). Since the results are based on a comparison between cultivars and areas, it makes more sense to include compounds that show variations between samples rather than compounds that are odour active but their concentration is similar among samples. Finally the GCO sensory evaluation had a limited number of judges and the identification of odour active peaks was not performed simultaneously with GC-MS analysis allowing the direct identification of odour active constituents by their MS spectra. As a result it is possible that some compounds were not detected or not identified properly leading to confusion in the GCO results.

With respect to the first PLSR model (Figure 12) the following relationships between chemical compounds and sensory descriptors are found. As suggested in the volatile discussion part the distinctive black pepper aroma and flavour of the Regent Rosnaes sample seems to derive from a number of grape derived compounds including terpenes (Citronellol, a-amorphene, a-muurolene, cadalin, a terpineol) and TDN as well as ethyl 9-decenolate and 2-pentanol. The plums raisins flavor which is perceived characteristically high in the Cabernet Cortis Pometet seems to mainly derive from 5 esters (ethyl octanoate, 2-methylpropyl acetate, butyl acetate, ethyl 3-phenylpropanoate, 2-phenylethyl acetate) as well as glycerol, propyl lactate and 3-methylpentan-1-ol. This agrees with the assumption made in the volatile discussion part that suggested that the high production of esters could contribute to the multitude of fruity sensory descriptors that are used to describe this variety. Moreover, the presence of glycerol, which is a sweet compound, could have enhanced the fruity aroma of plums and raisins since there is a cognitive link between sweetness and fruitiness (Zamora & Guirao, 2002). Tobacco odour seems to derive from 3-methyl cyclohexene, 3-pentanol, 1-propanol, styrene, ethyl heptanoate, ethyl (z)-3 hexenoate and 2-heptanol while smoked flavour from ethyl decenoate ethanol and (E)-2 Hexen-1-ol. Moreover odour red berries seems to be directly correlated to 4-methyl pentanol and 2,4 hexadiene. It should be kept in mind that plums raisins flavour, red berries odour, tobacco odour and smoked flavour are highly correlated in the plot, a pattern that applies also for the corresponding volatile compounds (Figure 12). As a result the Plums/raisins flavour, tobacco odour and smoked flavour are possible to derive from variations in the concentration of the sum of the aforementioned compounds which are located near these descriptors (Figure 12). Moreover the high amount of esters, which are used to explain these descriptors, furthermore proves that the high production of esters gives the unique characteristics of the Cabernet Cortis variety since all these descriptors are used to describe this cultivar from a sensory perspective.

Concerning the Odour and flavour animal/leather which are more common to the Bolero samples they seem to derive from tartaric acid, ethyl 2-phenylacetate and butan-2,3-dione, ethyl benzoate ethyl furan-carboxylate, respectively. Moreover acetaldehyde is projected close to leather animal odour. As suggested in the volatile discussion part, acetaldehyde could be partly responsible for the off-odour and therefore the off-flavour detected in the Bolero samples. Moreover the presence of tartaric acid close to animal leather odour suggests that this constituent could have played a major role to the development of unfavourable aroma/flavour since tartaric acid contributes with an sharp unpleasant taste to wine, especially when found in high concentrations.
Bitter taste, sour taste as well as astringent sensation are not placed close to any immediate descriptors, with the exception of reducing sugar. This result is expected since as mentioned earlier no chemical variables that could explain those attributes are included in the analysis. Finally sweet taste is not closely correlated with any immediate descriptors in the plot. A relative correlation can be found with fructose and as well as 4 esters (hexyl acetate, ethyl heptenoate, 3 methylbutyl acetate and ethyl hexenoate), if we project the position the sweet taste descriptor in the space between the 50%-100% ellipse. These compounds indeed describe the sweet taste since the sweet taste of fructose can be furthermore enhanced by the presence of fruity esters. The low correlation of the sweet descriptor with the aforementioned compounds is possibly a result of the low values sweet taste scored indiscriminately among the whole set of samples. This could have resulted to the low correlation pattern of sweet taste.
6. Conclusion
The comparative study between grape varieties cultivated in Denmark among different production sites resulted in several interesting findings which are described in the following paragraphs.

The Regent variety seems to be primarily characterized by a black pepper aroma and flavour while the resulting sensory quality of this wine is highly dependent on the cultivation area of the grapes. The odour and flavour of black pepper seem to originate mainly from grape derived compounds (terpenes and TND), which act synergistically to produce the sensory characteristics of Regent. The Rosnaes area seems to enhance in a great extend the black pepper aroma and flavour of the cultivar. On the other hand the Naestved sample exhibits less perceived intensities for the majority of the sensory descriptors, included in this study, with the exception of astringency. The difference in the sensory quality of the Regent wines could be attributed to the microclimate of the areas, which favours in the case of Naestved grape maturation and therefore results to higher number of terpene and TDN bound forms which are not odour active.

The varietal aroma of the Cabernet Cortis variety seems to derive from ester compounds. These compounds interact and create the distinctive quality of the Cabernet Cortis variety. The Cabernet Cortis sensory quality seems to be less dependent on the area production, since both areas produce wine with intense bitter, sour and astringent characteristics and have several similar aroma and flavour characteristics. Still the production site affects in some extent the aroma and flavour of this variety. The Pometet area enhances the black and red berries odour as well as plums/raisins flavour considerably for this cultivar which could be attributed to the higher concentration of esters found in this sample as well as the variability of the fruity aromas these esters contribute.

Concerning the Bolero variety, its distinctive characteristic from a sensory perspective is an animal/leather flavour and odour which can be unpleasant when found in high intensity. Both areas seem to produce wines which are perceived as having quite similar qualities. Still, the production site plays a role since the animal/leather quality is enhanced in the Rosnaes site giving an unfavourable character to the wine. This off odour flavour could be mainly explained by the presence of higher concentrations of tartaric acid and acetaldehyde.

Rondo seems to be a balanced variety from a sensory perspective, which can be used in blends as a base wine mixed with varieties with more notable characteristics to create high quality wines with distinctive character. Moreover Rondo seems to be less affected by the micro climate of the production area when compared to the rest cultivars included in this study. This could be used as an advantage when the aim is to produce wine with a steady sensory in Denmark where the microclimate of the cultivation areas varies greatly.

The aforementioned results reveal that not only the grape cultivars exhibit unique sensory characteristics but also that the production site plays an important role in enhancing/suppressing the aroma and flavour of each cultivar. The sensory wine quality of Regent is enhanced in the Rosnaes site of production site while the same site has the opposite effect for the Bolero variety. Rondo aroma and flavour seems to not be affect in
a great extent by cultivation area. Therefore it is possible to improve the sensory quality of wines in Denmark by choosing the appropriate production site for a variety to reach its full potential.
References


Bentzen, J. & Smith, V. (2009) Wine production in Denmark Do the characteristics of the vineyards affect the chances for awards?


Characterization of aroma and flavour profile of red cultivars from Denmark


Moulton, G., Miles, C., King, J., Echlin, C. & NWREC, W.M.V. Wine Grape Cultivar Trials 2000-2008 in the Cool Maritime Climate of Western WA.


