POLYPHENOL CHARACTERIZATION OF RED WINE FROM GERMANY'S WÜRTTEMBERG REGION

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Württemberg is Germany's fourth-largest winegrowing region and the only one with predominance of red varieties. To date, its wines have been poorly described with respect to physicochemical composition, including phenolic content, which has become of increasing interest to the industry, given the association of polyphenolics with desirable health benefits and the need for wine regions to identify points of differentiation. In this study, we sought to characterize a sample of commercial red wines from Württemberg with particular reference to their polyphenol composition. 37 red wines were assessed for general chemical composition (FTIR), mean degree of polymerization (mDP; HPLC), proanthocyanidins (HPLC) and total phenolic content (Folin-Ciocalteu). Large differences were found with regard to polyphenol content with wines made from *V. vinifera L.*, cv. 'Trollinger' showing the lowest and cuvées showing the highest concentrations. Wines made from cv. 'Pinot noir' contained the highest concentration of monomeric tannins, which may account for the harsh and bitter sensations sometimes anecdotally reported for this variety. In contrast, Trollinger wines had the highest proportion of polymeric tannins (trimers and above). Despite these differences, the mean degree of polymerization was approximately 3 for all varieties. This finding agrees with the thiolysis results, which showed that the ratio between terminal and extension units of the tannins was around 1:2, confirming a medium chain length of 3. These results may be used to inform production decisions, help optimize the quality of German wine, and assist in the branding and marketing of wines from Württemberg. Additionally, it may benefit longer-term goals of better understanding the key drivers of bitterness and mouthfeel sensations elicited by red wines.
Keywords: polyphenol; Trollinger; Pinot noir; polymerisation; thiolysis

Die Charakterisierung von deutschen Rotweinen aus der Region Württemberg. Württemberg ist die viertgrößte Weinbauregion in Deutschland und stellt die einzige Region dar, in der rote Rebsorten dominieren. Bis zum heutigen Tag sind die Weine der Region in Bezug auf ihre chemische Zusammensetzung, einschließlich der Polyphenole, jedoch nur unzureichend charakterisiert. Gerade letztere sind aber für die Industrie mehr und mehr von Interesse, da Polyphenole positive Auswirkungen auf die Gesundheit haben können und die Weinregionen immer mehr nach Möglichkeiten suchen, sich von anderen Regionen in der Zusammensetzung ihrer Weine zu unterscheiden. In dieser Studie haben wir versucht, verschiedene Rotweine aus Württemberg mit besonderem Fokus auf ihre Polyphenolzusammensetzung zu charakterisieren. Insgesamt wurden 37 Weine auf ihre allgemeine chemische Zusammensetzung (FTIR), ihren mittleren Polymerisationsgrad (mDP; HPLC), Proanthocyanidine (HPLC) und Gesamtphenolgehalt (Folin-Ciocalteu) untersucht. In Bezug auf den Gesamtphenolgehalt ergaben sich große Unterschiede, wobei Weine der Rebsorte 'Trollinger' die niedrigsten und Cuvée-Weine die höchsten Gehalte aufwiesen. Weine der Rebsorte 'Spätburgunder' (Pinot noir) zeigten die höchsten Gehalte an monomeren Tanninen, die für die harten und bitteren Noten verantwortlich sein könnten, die die Weine dieser Rebsorte manchmal besitzen können. Im Gegensatz dazu

zeigten die Trollinger-Weine den höchsten Anteil an polymeren Tanninen (Trimere und größer). Trotz all dieser Unterschiede lag der mittlere Polymerisationsgrad (mDP) über alle Rebsorten hinweg bei ungefähr 3. Dieses Ergebnis zeigt gute Übereinstimmung mit den Ergebnissen der Thiolyse, in der ein Verhältnis von 1:2 zwischen endständigen Tanninen und den entsprechenden Verlängerungseinheiten ermittelt wurde, was die mittlere Kettenlänge von 3 erklärt. Die Ergebnisse können als Hilfe bei der Herstellung dienen, um die Qualität deutscher Weine zu optimieren und die Weine aus Württemberg besser zu vermarkten. Zusätzlich kann auf lange Sicht ein besseres Verständnis der Auslöser von Bitterkeit und ähnlichen Geschmackseindrücken in Rotwein gewonnen werden.
Schlagwörter: Polyphenol; Trollinger; Spätburgunder; Polymerisation; Thiolyse

WÜRTTEMBERG

Currently, in Germany there are approximately 100,000 ha of vineyards located in 13 designated winegrowing areas. Württemberg is the fourth-largest winegrowing region within Germany, comprising about 11,000 ha. For several reasons, Württemberg can be regarded as unique: as in Pfalz (Palatinate) and Rheinhessen, Württemberg's average yield per hectare is around 100 hl/ha, considerably higher than that of other German regions. Furthermore, Württemberg has the highest proportion of red varieties at approximately 60 %; in all other regions, white grapes predominate (Deutscher Wein Statistik, 2014). Also in Württemberg, most of the vintners are organized in cooperatives. Although the number of cooperatives has declined significantly since 1996, they are still responsible for approximately 70 % of the total winegrowing area (Honold, Weinbaukartei Weinsberg, pers. communication).

Red wines from Württemberg are well-known for their easy-drinking style. This is partially due to the varieties typically grown in this region: 'Trollinger', 'Pinot noir', 'Lemberger', and 'Pinot meunier'. Anecdotally, these varieties do not reach full physiological ripeness in Württemberg, especially with regard to phenols. Most of the red grapes undergo flash pasteurization (82 °C for 2 to 3 min) to achieve fruit-driven, low polyphenol-containing wines (Jakob et al., 1997). Classical mash fermentation is also performed, but to a much lower extent. These wines are typically used as a component in cuvée blends to add structure to the aforementioned flash pasteurized wines (Blankenhorn, 2002).

During the last 10 to 15 years several efforts have been taken to raise the quality of red wine. Local varieties have been partially replaced by internationally known varieties, such as 'Merlot', 'Syrah', 'Cabernet Sauvignon', and 'Cabernet Franc'. Winemaking technology has incorporated procedures such as fermentation and ageing in barriques, and post-fermentative extraction of the grapes. While in some cases the results have been very successful, polyphenol ripeness remains an issue. Even though wines might mature for 14 to 18 months in barrel after malolactic fermentation, they often show green, harsh astringency, anecdotally attributable to the tannin composition. Additionally, wine colour tends to show brown hues relatively early. Several processing technologies, including oxygen management and pressure swing adsorption, have been tested to deal with these issues, with mixed success (Blankenhorn and Plag, 2006; Blankenhorn and Hirt, 2005).

POLYPHENOLS

Of particular interest in recent years has been the elucidation of the polyphenol composition of red wine. This has come from two sources: firstly, increased interest in the health-promoting properties of these compounds, which have been linked to lower rates of coronary heart disease (Iriti and Varoni, 2015), anti-cancer properties, including inhibition of human cancer cells (Grønbaek et al., 2000; Barron et al., 2014), and even increased longevity in some animal studies (Aires et al., 2012). Therefore, elucidating the polyphenolic composition of a region's red wines offers a potential point of differentiation in a competitive international market place.

Secondly, polyphenols are also of increasing interest because of their association with bitterness and astringency, two nominally aversive sensations linked with consumer rejection of foods and beverages (Fischer and Sokolowsky, 2011; Sokolowsky and Fischer, 2012), yet-at moderate levels-considered part of the typicity of many red wine styles (Lesschaeve and Noble, 2005; Bajec and Pickering, 2008). As well as helping to differentiate wine types (Schneider, 2000), they are also strongly associated with the richness or "body" of red wine (Llaudy et al., 2004). However, over-extraction of polyphenols during red wine making leads to bitter and harsh wines (Weber et al., 2013); indeed, the 'art' of fine red wine making is largely about the careful extraction and fractionation of grape-derived phenolic components.

SPECIES

The polyphenol component of red wine is comprised of tannins, catechins, and anthocyanins (Cheynier et al., 2006; Fischer and Noble, 1994; Lea et al., 1978; VIDAL et al., 2004). Monomeric flavan-3-ols are generally perceived more bitter than higher oligomers or polymers (Hufnagel and Hofmann, 2008; Waterhouse, 2002). Up to a chain length of four units (i.e., tetramers) bitterness is typically predominant over astringency (Thorngate and Noble, 1995). Bitterness and astringency are not only influenced by the degree of polymerization, but also by the degree of galloylation. Tannins extracted from the skins have been classified as less astringent than seed tannins with a higher level of galloylation (Cheynier et al., 2006; Souquet et al., 1996). Cheynier et al. (2006) also found that the ratio of the concentration of polyphenols and anthocyanins has a great influence on bitterness. If not enough anthocyanins are available, oligomers polymerize. Maybe this is the reason for the saying that only tannic wines can age well (Llaudy et al., 2004). Due to the lack of aging, young red wines still contain a lot of short-chain phenols. They are usually rather bitter and astringent and, therefore, also referred to as "hard". The longer tannin chains of aged wines are accordingly characterized as "soft" (Noble, 1994). Furthermore, there are also differences in the sensory characteristics of the stereoisomers (+)-catechin and (-)-epicatechin; epicatechin is more bitter than catechin, and the bitterness and astringency on the palate is longer (Drewnowski and Gomez-Carneros, 2000; Noble, 1994).

VITICULTURAL AND OENOLOGICAL CONSIDERATIONS

In the early stages of grape berry development exclusively colourless polyphenols are synthesized by the grapevine to protect the berries from potential predators and various fungi (Ehrhardt et al., 2014). At veraison – the stage when coloration of red varieties begins - anthocyanins are produced, largely as a response to intense UV-irradiation during the hot summer month of August (Barnud et al., 2014). Several viticultural interventions have been identified as impacting the final polyphenol content of the grape. For instance, reduced crop levels and less shading through removal of leaves can increase final phenol content (Fox and Pour Nikfardjam, 2008). Additionally, stress factors such as mechanical cluster thinning and fungal pressure increase phenol content (Pour Nikfardjam and Strauss, 2011; Strauss et al., 2013).

Most polyphenols in wines are extracted from the grapes during winemaking (Cadot et al., 2012). Hence, the final content of polyphenols greatly depends on the winemaking technique used (Gabetta et al., 2000; Revilla et al., 1991). Early in vinification, it is mainly polyphenols from the skins that are extracted. With increasing maceration time, seed tannins play an increasingly important role as a source of polyphenols (Shi et al., 2005). Seed tannins are more polymeric than those derived from the skin, and thus contribute to a large extent to the wine's bitterness and astringency (Curko et al., 2014). They are extracted by ethanol during must fermentation, whereas temperature is a crucial element in the case of thermovinification (Jakob et al., 1997). The latter technique favours colour (anthocyanin) extraction, and – since ethanol is not available at this early stage – avoids contemporaneous extraction of the harsher and more astringent tannins from the seeds (Sacchi et al., 2005).

Several attempts have been made to reduce bitterness and astringency in wine using various fining agents with varying success (Bonerz et al., 2004; Pour Nikfardjam et al., 2006; Tschiersch et al., 2010). The legal requirement to label potential allergens has also changed the use of these winemaking additives (Schneider 2014; Schümann et al., 2013). For instance, an increasing number of fining agents from non-allergenic sources are being used in industry (Kammer et al., 2012; Tschiersch et al., 2010), potentially altering polyphenols composition and altering the sensory profile.

STUDY OBJECTIVES

Given this interest in the polyphenolic content of red wine, and the increasing need for wine regions to identify points of differentiation in their products, our main objective was to characterize a representative sampling of commercial red wines from the Württemberg region, with particular reference to their polyphenol composition. Such a characterization may assist in branding and marketing, and also benefit longer-term goals of better understanding the key drivers of bitterness and mouthfeel sensations elicited by red wines. This information can be used to inform production decisions and help optimize the quality of German wine.

MATERIALS AND METHODS

WINE SAMPLES

A total of 37 red wines made from different varieties, 'Lemberger' (n = 12), 'Pinot noir' (n = 5), 'Trollinger' (n = 10), and cuvée (n = 10) from the vintages between 2009 and 2011 was analysed. The wines were provided by local wineries based on their experience of these wines eliciting high astringency or bitterness compared to their other products.

CHEMICALS AND STANDARDS

All solvents were of high-performance liquid chromatography (HPLC) grade. Acetone, acetic acid, dichloromethane, ethanol, n-hexane, and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Acetonitrile was purchased from Carl Roth (Karlsruhe, Germany), benzyl mercaptane from AlfaAesar (Karlsruhe, Germany), and methanol was purchased from VWR International (Leuven, Belgium).
Folin-Ciocalteu's phenol reagent was purchased from Merck (Darmstadt, Germany).
(+)-Catechin, (-)-epicatechin, and (-)-epicatechin gallate were purchased from Carl Roth (Karlsruhe, Germany) and Procyanidin B2 was purchased from Extrasynthese (Genay, France).

HPLC ANALYSIS

THIOLYSIS AND MEAN DEGREE OF POLYMERIZATION (mDP)

Analysis was performed using the method by Bonerz (2003). Briefly, 400 µl of wine were added to 400 µl of a 5 % solution of benzyl mercaptane in acidified methanol (0.2 M HCl) in an HPLC vial. The vial was sealed and placed for 4 min in a boiling water bath. After cooling to room temperature the obtained solution was injected into an HPLC (UltiMate 3000, Dionex, Idstein, Germany) equipped with an autosampler and a diode-array detector. Separation of compounds was achieved using a LiChrospher 100 RP-18, 250 x 4.6 mm, 5 µm column (VWR, Darmstadt, Germany) and a pre-column of the same material maintained at 25 °C. The chromatograms were recorded at 280 nm, and identification and quantification of the terminal and extension units were carried out using diode array detection spectra and calculated as (+)-catechin, (-)-epicatechin and (-)-epicatechin gallate with three seperate calibration curves. The mDP values were calculated according to the method published by Atanasova et al. (2002). The injection volume was 20 µl.

PROANTHOCYANIDINS

Analysis was performed using the method by Pour Nikfardjam (2001) based on a method of Lazarus et al. (1999). Briefly, a C18-cartridge (Strata C18-E, 55 µm, 70 A, 500 mg, 6 ml, Phenomenex, Aschaffenburg, Germany) was conditioned with 5 ml methanol and water. After that 10 ml of wine were loaded onto the cartridge and interfering substances washed off with water. The cartridge was then dried under a gentle airflow. The proanthocyanidins were eluted using 5 ml acidified acetone (0.5 % acetic acid). The organic solvent was then evaporated at 35 °C under reduced pressure. The residue was re-dissolved in 1 ml acidified acetone and then subsequently used for analysis without any further pre-treatment. An HPLC (UltiMate 3000, Dionex, Idstein, Germany) equipped with an auto-sampler, diode-array and fluorescence detector was used. Separation of compounds was achieved using a LUNA-Silica, 250 x 4.6 mm, 5 µm (Phenomenex, Aschaffenburg, Germany) column and a pre-column of the same material maintained at 37 °C. Fluorescence spectra at an excitation wavelength of 276 nm and an emission wavelength of 316 nm were used to identify and quantify the contained proanthocyanidins expressed as (+)-catechin. Additionally recorded diode array spectra at 280 nm were used to check peak purity.

TOTAL POLYPHENOL CONTENT AND GENERAL COMPOSITION

The total polyphenol content was determined using the Folin-Ciocalteu method (Ritter et al., 1994) with (+)-catechin as standard. General components (density, alcohol content, extract, reducing sugar, glucose, fructose, pH-value, total acidity, tartaric acid, lactic acid, malic acid, and acetic acid, and glycerol) were measured on a WineScan FT-120 (Foss, Hillerod, Denmark) (Baumgartner et al., 2001). All analyses were performed in duplicate.

DATA ANALYSIS

All data analyses was carried out using Excel (Versions 2003 and 2010, Microsoft, Redmond, USA), XLStat (Version 2011.1.05, Addinsoft, Paris, Frankreich) and SPSS (Version 21, IBM, Armonk, USA). Analysis of variance (ANOVA) was conducted and Tukey's $HSD_{0.05}$ test was used for separation of means. Principal component analysis was conducted on all analytes.

RESULTS AND DISCUSSION

GENERAL COMPOSITION

Results of the FTIR analysis are given in Table 1. The mean alcohol content across all varieties equates to 13.2 % v/v, which is somewhat higher than the 12.0 to 12.5 % v/v, generally found in Württemberg, the lower levels reflecting consumer preference for easy-drinking red wines from this region (Württemberg Wine Quality Approval Board, personal communication). Trollinger wine shows lower levels of extract, likely reflecting the stylistic trend of higher residual sugar in this variety. It also has a lower concentration of tartaric acid compared with other varieties, concurring with anecdotal reports for this variety in Württemberg. This is consistent with the speculation that 'Trollinger' does not reach full ripeness in this region, tending to produce wines of more intense character and balance only in more southern European locales, such as South-Tyrol in northern Italy (Huber et al., 2005).

'Lemberger' was the variety with the lowest pH, associated with its higher total acidity values. Interestingly, volatile acidity levels are higher in cuvées and Pinot noir wine compared with Lemberger and Trollinger wines. This may be attributable to the fact that Pinot noir wines and cuvées are more likely to undergo maturation in barrels, which is known to increase volatile acidity (Croitoru, 2013).

There is also a large (2.6 x) difference between the varieties in total polyphenol content. 'Lemberger', previously identified as a polyphenol-rich variety (Strauss et al., 2013), had similar contents to 'Pinot noir'. In contrast, 'Trollinger' shows the lowest polyphenol values, in agreement with other phenolic results presented below. The high values for the cuvées are probably due to the use of anthocyanin-rich varieties such as 'Cabernet Dorsa' or 'Cabernet Mitos' as blending partners, which may contribute a significant portion of phenols (Schiefer and Steinbrenner, 2012).

PROANTHOCYANIDINS

The results of NP-HPLC on tannic composition are given in Figure 1 and expressed as mg catechin/l. Pinot noir wine shows significantly higher concentration of both monomeric and total tannins, consistent with some reports from other wine regions (Kemp et al., 2011). While this finding may be positive with respect to the potential health benefits of Pinot noir wines from Württemberg, it is a variety well-known to produce wines with harsh, green, and astringent tannins when grapes do not reach complete ripeness (Lafontaine et

Table 1: Basic physicochemical composition of wines measured by FTIR and Folin-Ciocalteu method (total phenols). Data represent mean ± standard deviation; wines with different letters differ significantly for each parameter ($p < 0.05$ from ANOVA followed by Tukey's HSD test)

Parameter	Cuvée (n = 10)	Lemberger (n = 12)	Pinot noir (n = 5)	Trollinger (n = 10)
Density	0.9941 ± 0.0010 *a*	0.9932 ± 0.0017 *a*	0.9936 ± 0.0014 *a*	0.9931 ± 0.0024 *a*
Alcohol (g/l)	106.9 ± 2.8 *a*	104.7 ± 3.1 *a*	103.8 ± 8.3 *a*	102.6 ± 3.6 *a*
Extract (g/l)	30.1 ± 2.7 *a*	27.2 ± 4.1 *a*	27.7 ± 5.2 *a*	25.8 ± 5.2 *a*
Residual sugar (g/l)	2.8 ± 1.2 *a*	3.8 ± 2.3 *a*	3.8 ± 1.5 *a*	5.2 ± 5.4 *a*
Glucose (g/l)	0.0 ± 0.5 *a*	0.8 ± 1.3 *a*	0.6 ± 0.6 *a*	1.7 ± 2.7 *a*
Fructose (g/l)	0.5 ± 0.8 *a*	1.0 ± 1.1 *a*	1.0 ± 0.8 *a*	1.8 ± 3.0 *a*
pH	3.85 ± 0.11 *a*	3.59 ± 0.08 *b*	3.76 ± 0.21 *ab*	3.69 ± 0.13 *b*
Total acidity (g/l)	5.0 ± 0.5 *a*	5.0 ± 0.4 *a*	4.5 ± 0.4 *ab*	4.4 ± 0.5 *b*
Tartaric acid (g/l)	2.1 ± 0.3 *a*	2.0 ± 0.3 *a*	1.9 ± 0.3 *a*	1.4 ± 0.2 *b*
Lactic acid (g/l)	2.5 ± 0.5 *a*	1.9 ± 0.3 *b*	2.2 ± 0.1 *ab*	2.0 ± 0.8 *ab*
Malic acid (g/l)	0.2 ± 0.3 *a*	0.2 ± 0.2 *a*	0.3 ± 0.1 *a*	0.7 ± 0.8 *a*
Volatile acidity (g/l)	0.73 ± 0.18 *a*	0.53 ± 0.16 *bc*	0.70 ± 0.11 *ab*	0.44 ± 0.13 *c*
Glycerol (g/l)	8.2 ± 1.0 *a*	8.2 ± 0.8 *a*	7.7 ± 0.9 *a*	7.2 ± 0.8 *a*
Total phenols (mg/l)	2940 ± 829 *a*	1975 ± 631 *b*	2074 ± 458 *ab*	1121 ± 259 *c*

al., 2013), or through over-extraction of the grape seeds post-alcoholic fermentation (Weiand, 2009). Thus, optimisation and careful management of Pinot's polyphenol potential is important, and includes several systems to remove the seeds from fermenters to allow extraction of the skins alone, and removal of seeds from the bottom of tanks after alcoholic fermentation is completed (Weiand, 2009).

Lemberger wines and cuvées show very similar tannic composition. This may reflect the common use of Lemberger wine as the base wine in cuvées from this region, with the final wine typically blended with other preferred varieties such as Trollinger and Pinot noir wines.

Pinot noir and Trollinger wines show very similar relative tannic composition, with approximately two thirds of the tannins composed of monomers, 22 % of dimers, and 7 % trimers. However, 'Trollinger' is the variety with the lowest total tannin content, with, for instance, only 40 % of the total concentration of 'Pinot noir'. This is often reflected in the generally lighter-bodied style and colour of reds from this variety (Eder et al, 2004). Interestingly, Trollinger wine contains the highest proportion of polymeric tannins (trimers and larger), which make up 14.9 % of its total tannic fraction. This might be due to the generally lower resistance of Trollinger wines to oxidative polymerization of the tannins (Fischer, 2003). Thus, wines made from 'Trollinger' showed the most progressed ageing with regard to tannin polymerization.

Overall, hexamers and heptamers make up only a very small fraction of the tannin composition of these wines, perhaps reflecting the relatively recent vintages used in the study. Pinot noir wines tended towards the highest concentration of these larger polymers.

MEAN DEGREE OF POLYMERIZATION (mDP)

Overall, the mDP values indicate that these wines are generally of medium chain-length and do not differ between varieties to a large extent (Fig. 2). Mean values for mDP vary between 2.6 (Lemberger) and 3.1 (Trollinger). The highest mDP values were found in Trollinger, which was significantly higher than in Lemberger (standardised difference = 3.689, critical value = 2.705, $p < 0.004$). 'Trollinger' is known as a variety poor in polyphenols and thus tends to show brown hue and oxidative flavours relatively early in its development (Eder et al., 2004). During oxidation, polyphenols form longer chains and hence mDP increases (Gonzalez-Manzano et al., 2006). All other varieties showed no significant difference with regard to mDP values. Regardless of origin or variety, mDP generally increases with wine age and is faster if anthocyanin content is higher, because anthocyanins are also able to polymerize with procyanidins (Vidal et al., 2002).

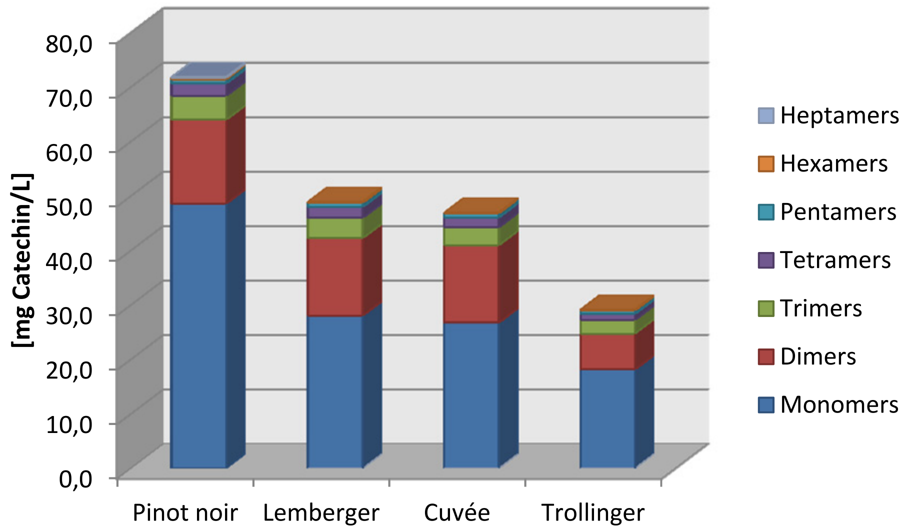


Fig. 1: Proanthocyanidin composition derived from Normal-Phase HPLC analysis (Pinot noir, n=5; Lemberger, n=12; Cuvée, n=10; Trollinger, n=10)

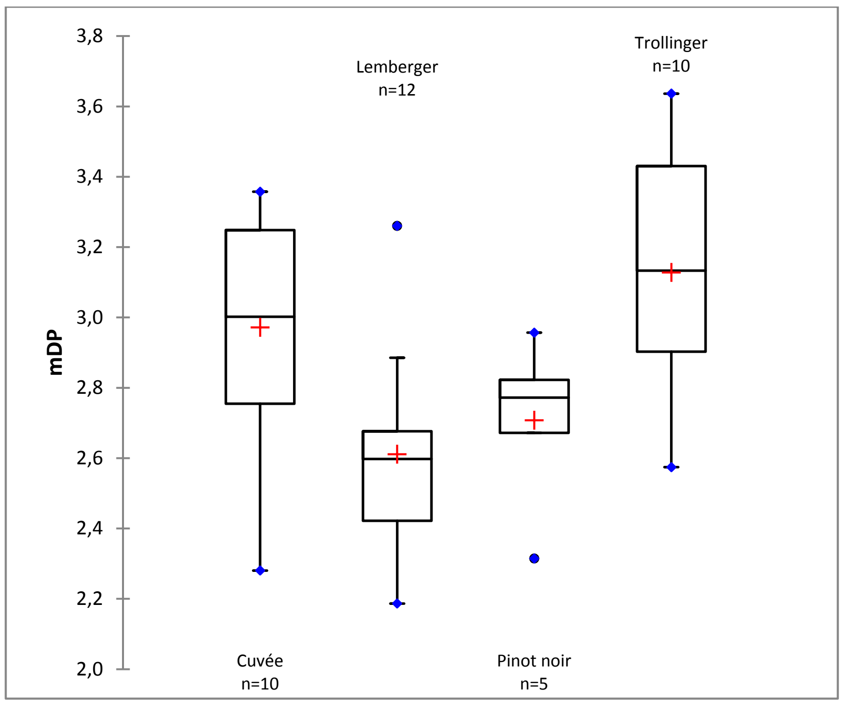


Fig. 2: Results of the analysis on mean degree of polymerisation (mDP) of the polyphenolic fraction

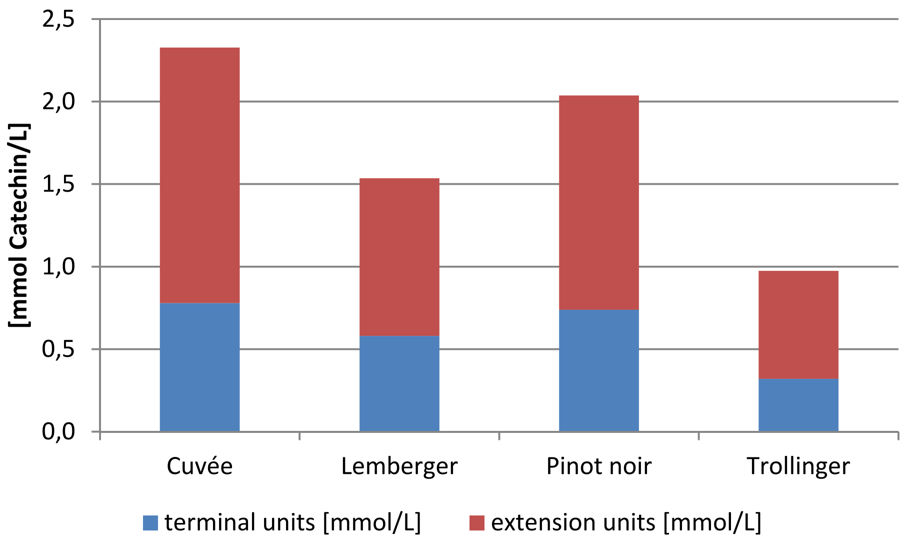
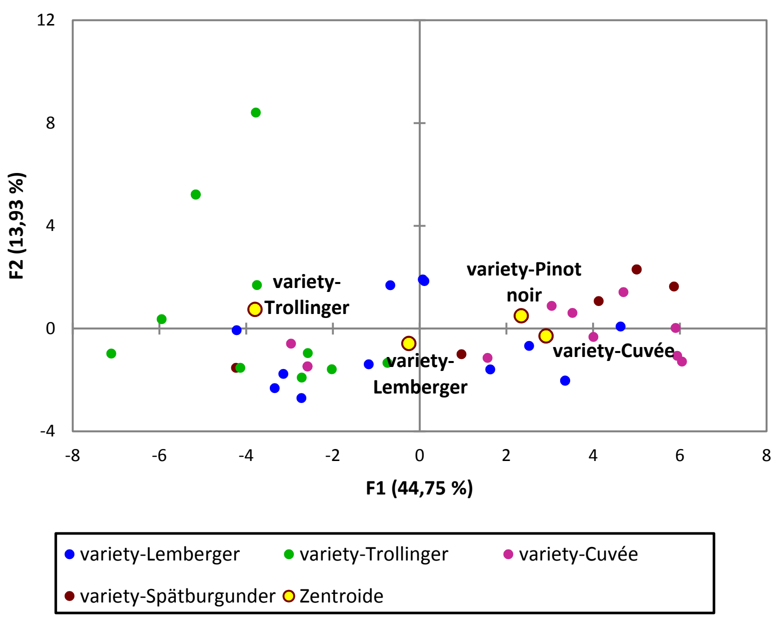
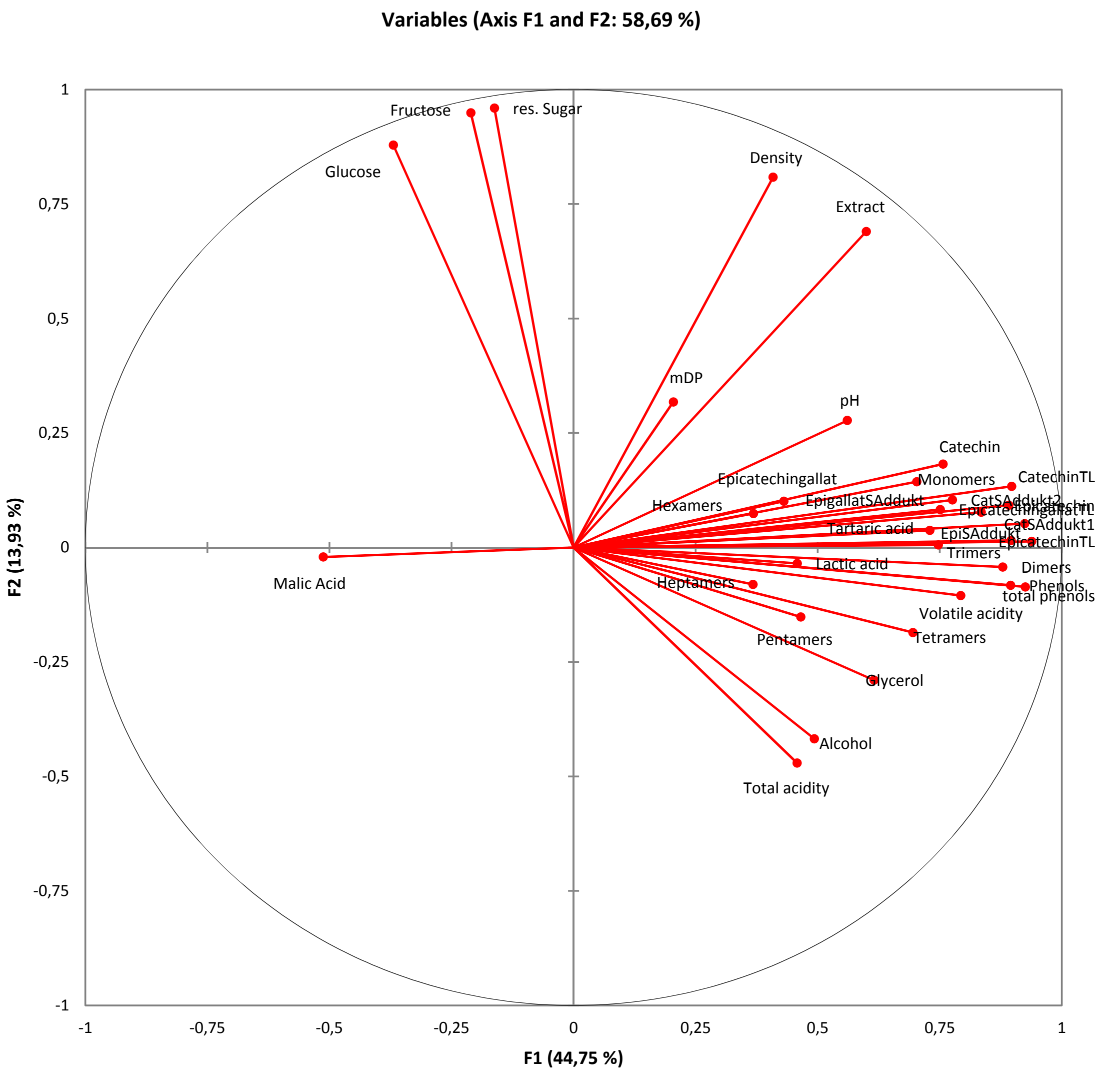


Fig. 3: Proanthocyanidin composition derived from thiolysis and subsequent HPLC analysis (Pinot noir, n=5; Lemberger, n=12; Cuvée, n=10; Trollinger, n=10)



a)

Fig. 4: Principal component analysis (PCA) on all analytes with regard to the separation of varieties



b)

Fig. 4: Principal component analysis (PCA) on all analytes with regard to the separation of varieties

THIOLYSIS

Figure 3 shows the results from the thiolysis. Cuvées show the highest content of tannic compounds, which is mainly due to their high polyphenol content in general (see FTIR results; Table 1). Generally, the concentration of tannic compounds shows very good correlation with the phenol content, as measured by FTIR ($R^2 = 0.946$). Interestingly, the ratio between terminal and extension units is very close to 1 : 2 across all varieties. This supports the results from the mDP values as they are close to a value of 3 (see above).

The separation of the chain units by HPLC before and after thiolysis revealed that catechin and epicatechin monomers occur in wine on average in equal concentrations, but monomers were especially high in Pinot noir wines. Catechin occurred mainly as a terminal unit, and epicatechin and epicatechin gallate rather as extension units (data not shown). This is consistent with the results of Souquet et al. (2000) and Sarni-Manchado et al. (1999).

PRINCIPAL COMPONENT ANALYSIS (PCA)

A PCA was run on all analytical data to further assess the influence of the single parameters on the separation between varieties, and elucidate the relationship between the analytes themselves. Results show that the varieties are primarily separated along PC1 (Fig. 4a), which is largely defined as a phenol vs. malic acid dimension (Fig. 4b). The separation of Trollinger wines from other varieties along PC1 is largely due to its higher mean values for malic acid, indicative of wines that did not undergo full malolactic fermentation. Little separation between wines is observed along PC2, which accounts for just 14 % of the variation, and is mainly defined as a sugar and density/extract-related axis. Amongst the analytes themselves, alcohol was negatively associated with reducing sugars, as expected; however, it did not associate with either total extract or the mean degree of polymerization of the tannins. The various polyphenolic species were all positively and highly correlated, suggestive of both commonality in the extraction and retention behaviours of these components in wine, and also perhaps of some redundancy in the analyses performed.

LIMITATIONS, FURTHER RESEARCH AND CONCLUSIONS

The wines used here were not made by standardized vinification procedures and represented multiple vintages. Furthermore, a sampling bias exists with respect to the wines selected; thus, the differences in polyphenol composition should be viewed as tentative. For 'Pinot noir', only five wines were analysed; a greater number of samples would increase confidence in the findings reported. More research is encouraged to further elucidate the link between polyphenolic composition and perceptions of astringency and bitterness, both with respect to further defining Württemberg wines, and the more general goal of understanding composition/sensory relationships. In conclusion, we have applied modern analytical techniques to successfully characterize the phenolic and general composition of representative red wines from Württemberg. While this information may assist German vintners now with production decisions, we support further research aimed at optimising the positioning of Württemberg wines within a very competitive marketplace. Points of differentiation and marketing foci may include health and taste advantages, for which these findings provide some base-line indicators.

REFERENCES

Aires, D.J., Rockwell, G., Wang, T., Frontera, J., Wick, J., Wang, W., Tonkovic-Capin, M., Lu, J., Lezi, E., Zhu, H. and Swerdlow, R.H. 2012: Potentiation of dietary restriction-induced lifespan extension by polyphenols. Biochim. Biophys. Acta 1822(4): 522-526

Atanasova, V., Fulcrand, H., Cheynier, V. and Moutounet, M. 2002: Effect of oxygenation on polyphenol changes occurring in the course of winemaking. Anal. Chim. Acta (458): 15-27

Bajec, M. and Pickering, G.J. 2008: Astringency: mechanisms and perception. Crit. Rev. Food Sci. Nutr. 49(9): 858-875

Barnuud, N.N., Zerihun, A., Gibberd, M. and Bates, B. 2014: Berry composition and climate: responses and empirical models. Int. J. Biometeor. 58(6): 1207-1223

Barron, C.C., Moore, J., Tsakiridis, T., Pickering, G. and Tsiani, E. 2014: Inhibition of human lung cancer cell proliferation and survival by wine. Cancer Cell Int. 14: 6.

Baumgartner, D., Bill, R. und Roth, I. 2001: Traubenmostanalyse mit Hilfe der FTIR-Spektroskopie. Schw. Z. Obst- und Weinbau 137(2): 46-48

Blankenhorn, D. 2002: Thermische Verfahren zur Rotweinbereitung. Dt. Weinbau (16): 24-28

Blankenhorn, D. und Hirt, T. 2005: Rotweinbereitung: Spätburgunder wird mit Druck besser. Rebe und Wein 58(10): 14-16

Blankenhorn, D. und Plag, P. 2006: Sauerstofftherapie für gerbstoffbetonte Rote. Rebe und Wein 59(11): 21-23

Bonerz, D. 2003: Influence of the fining agent CFine on colour, antioxidative capacity and phenolic compounds in Pinot noir wine (Project report). Canterbury (NZ): Lincoln Univ. Centre Vitic. Oenol., 2003

Bonerz, D.P.M., Bloomfield, D.G., Dykes, S.I., Rawel, H.M., Rohn, S., Kroll, J., Creasy, G.L. and Pour Nikfardjam, M.S. 2004: A new gentle fining agent for Pinot noir. Mitt. Klosterneuburg 54(3/4): 86-95

Cadot, Y., Caille, S., Samson, A., Barbeau, G. and Cheynier, V. 2012: Sensory representation of typicality of Cabernet franc wines related to phenolic composition: Impact of ripening stage and maceration time. Anal. Chim. Acta (732): 91-99

Cheynier, V., Duenas-Paton, M., Salas, E., Maury, C., Souquet, J.M., Sarni-Manchado, P. and Fulcrand, H. 2006: Structure and properties of wine pigments and tannins. Am. J. Enol. Vitic. 57: 298-305

Croitoru, C. 2013: The influence of malolactic fermentation on the sensorial profile of red wines varieties. Bull. OIV 86(983/985): 93-102

Curko, N., Kovacevic Ganic, K., Gracin, L., Dapic, M., Jourdes, M. and Teissedre, P.L. 2014: Characterization of seed and skin polyphenolic extracts of two red grape cultivars grown in Croatia and their sensory perception in a wine model medium. Food Chem. 145: 15-22

Deutscher Wein Statistik, 2014: http://www.deutscheweine.de/fileadmin/user_upload/Website/Service/Downloads/statistik_2014_NEU_web.pdf (29.6.2015)!

Drewnowski, A. and Gomez-Carneros, C. 2000: Bitter taste, phytonutrients, and the consumer: a review. Am. J. Clin. Nutr. 72: 1424-1435

Eder, R., Oswald, B. und Wendelin, S. 2004: Einfluss von pektolytischen Enzympräparaten mit Acetylaseaktivität sowie Botrytisbefall und Maischeerhitzung auf Anthocyaninzusammensetzung und Qualität von Rotwein. Mitt. Klosterneuburg 54(7/8): 207-221

Ehrhardt, C., Arapitsas, P., Stefanini, M., Flick, G. and Mattivi, F. 2014: Analysis of the phenolic composition of fungus-resistant grape varieties cultivated in Italy and Germany using UHPLC-MS/MS. J. Mass Spectrom. 49(9): 860-869

Fischer, U. 2003: Zielgerichteter Ausbau von Rotweinstilen. Winzer-Zeitschrift 18(1): 26-29

Fischer, U. and Noble, A.C. 1994: The effect of ethanol, catechin concentration, and pH on sourness and bitterness of wine. Am. J. Enol. Vitic. 45: 6-10

Fischer, U. und Sokolowsky, M. 2011: Bitterer Geschmack im Weißwein. Dt. Weinmagazin (6): 10-14

Fox, R. und Pour Nikfardjam, M. 2008: Licht oder Schatten? Aspekte zur Entblätterung. Dt. Weinbau (10): 28-31

Gabetta, B., Fuzzati, N., Griffini, A., Lolla, E., Pace, R., Ruffilli, T. and Peterlongo, F. 2000: Characterization of proanthocyanidins from grape seeds. Fitoterapia 71(2): 162-175

Gonzalez-Manzano, S., Santo-Buelga, C., Perez-Alonso, J.J., Rivas-Gonzalo, J.C. and Escribano-Bailon, M.T. 2006: Characterization of the mean degree of polymerization of proanthocyanidins in red wines using liquid chromatography-mass spectrometry (LC-MS). J. Agric. Food Chem. 54(12): 4326-4332

Grønbaek, M., Becker, U., Johansen, D., Gottschau, A., Schnohr, P., Hein, H.O., Jensen, G. and Sørensen, T.I. 2000: Type of alcohol consumed and mortality from all causes, coronary heart disease, and cancer. Ann. Intern. Med. 133(6):411-419

Huber, E., Wendelin, S., Kobler, A., Behofer, E. und Eder, R. 2005: Bestimmung der Phenolzusammensetzung, der sensorischen Eigenschaften und der antioxidativen Kapazität im Reifeverlauf bei vier Südtiroler Rotweinsorten. Mitt. Klosterneuburg 55(1/2): 3-21

Hufnagel, J.C. and Hofmann, T. 2008: Orosensory-directed identification of astringent mouthfeel and bitter-tasting compounds in red wine. J. Agric. Food Chem. 56: 1376-1386

Iriti, M. and Varoni, E.M. 2015: Moderate red wine consumption in cardiovascular disease: Ethanol versus polyphenols. In: Preedy, V. and Watson, R.R. (eds.): The mediterranean diet. An evidence-based approach, p. 143-151. – London: Academic Press, 2015

Jakob, L., Hamatschek, J. und Scholten, G. 1997: Der Wein. 10. Aufl. Stuttgart: Ulmer, 1997

Kammer, K., Wallbrunn, C. und Dietrich, H. 2012: Einsatz als alternatives Schönungsmittel in der Weinbereitung? Chitosan. Dt. Weinmagazin (4): 16-21

Kemp, B.S., Harrison, R. and Creasy, G.L. 2011: Effect of mechanical leaf removal and its timing on flavan-3-ol composition and concentrations in Vitis vinifera L. cv. Pinot Noir wine. Austr. J. Grape Wine Res. 17(2): 270-279

Lafontaine, M., Stoll, M. und Schultz, H.R. 2013: Tannine und Anthocyane im Spätburgunder. Dt. Weinbau (8): 72-77

Lazarus, S.A., Adamson, G.E., Hammerstone, J.F. and Schmitz, H.H. 1999: High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages. J. Agric. Food Chem. 47: 3693-3701

Lea, A.G.H. and Arnold, G.M. 1978: The phenolics of ciders: bitterness and astringency. J. Sci. Food Agric. 29: 478-483

Lesschaeve, I. and Noble, A.C. 2005: Polyphenols: Factors influencing their sensory properties and their effects on food and beverage preferences. Am. J. Clin. Nutr. 81: 330-335

Lla udy, M.C., Canals, R., Canals, J.M., Rozes, N., Arola, L. and Zamora, F. 2004: New method for evaluating astringency in red wine. J. Agric. Food Chem. 52: 742-746

Noble, A.C. 1994: Bitterness in wine. Physiol. Behav. 56: 1251-1255

Pour Nikfardjam, M.S. 2001: Polyphenole in Weißweinen und Traubensäften und ihre Veränderung im Verlauf der Herstellung. PhD Thesis Univ. Giessen, 2001

Pour Nikfardjam, M. und Strauss, M. 2011: Ertragsregulierung mit dem Vollernter: Immer feste drauf! Dt. Weinmagazin (15): 13-15

Pour Nikfardjam, M., Schmidt, E. und Kuruczné, E. 2006: Bitterkeit und Adstringenz schönen? Dt. Weinbau (22): 22-25

Revilla, E., Bourzeix, M. and Alonso, E. 1991: Analysis of catechins and proanthocyanidins in grape seeds by HPLC with photodiode array detection. Chromatographia 31: 465-468

Ritter, G., Cliffe, S., Fawer, M.S., Maier, G. and Takata, K. 1994: Enzyme assays for the phenolic content of natural juices. J. Agric. Food Chem. 42: 1824-1828

Sacchi, K.L., Bisson, L.F. and Adams, D.O. 2005: A review of the effect of winemaking techniques on phenolic extraction in red wines. Amer. J. Enol. Vitic. 56(3): 197-206

Sarni-Manchado, P., Cheynier, V. and Moutounet, M. 1999: Interactions of grape seed tannins with salivary proteins. J. Agric. Food Chem. 47(1): 42-47

Schiefer, H.C. und Steinbrenner, P. 2012: Erfahrungen im Anbau: internationale rote Sorten. Dt. Weinbau (18): 22-25

Schneider, I. 2014: Allergene Stoffe im Wein gibt es eine Lösung? Teil I + II. Dt. Weinmagazin (12): 30-32; (13): 28-29

Schneider, V. 2000: Die Adstringenz der Rotweine Das gute Gefühl beim Trinken. Dt. Weinmagazin (18): 18-25

Schümann, C., Patzl-Fischerleitner, E., Schmuckenschlager, M. und Eder, R. 2013: Nachweisbarkeit von Ovalbumin, Casein und Lysozym nach Zugabe in Most und Wein in Abhängigkeit von Filtration und Bentonitschönung. Mitt. Klosterneuburg 63(2): 65-69

Shi, J., Nawaz, H., Pohorly, J., Mittal, G., Kakuda, Y. and Jiang, Y. 2005: Extraction of polyphenolics from plant material for functional foods-engineering and technology. Food Rev. Int. 21: 139-166

Sokolowsky, M. and Fischer, U. 2012: Evaluation of bitterness in white wine applying descriptive analysis, time-intensity analysis, and temporal dominance of sensations analysis. Anal. Chim. Acta (732): 46-52

Souquet, J.M., Cheynier, V., Brossaud, F. and Moutounet, M. 1996: Polymeric proanthocyanidins from grape skins. Phytochemistry 43: 509-512

Souquet, J.M., Labarbe, B., Le Guerneve, C., Cheynier, V. and Moutounet, M. 2000: Phenolic composition of grape stems. J. Agric. Food Chem. 48: 1076-1080

Strauss, M., Nikfardjam, M.P. und Nöbel, B. 2013: Ertragsregulierung im Heckenschnitt: Äußerer Einfluss und innere Werte. Rebe und Wein 66(7): 24-27

Thorngate, J.H.I. and Noble, A.C. 1995: Sensory evaluation of bitterness and astringency of 3R(-)-epicatechin and 3S(+)-catechin. J. Sci. Food Agric. 67: 531-535

Tschiersch, C., Nikfardjam, M.P., Schmidt, O. und Schwack, W. 2010: Entwicklung neuer Weinschönungsmittel zur gezielten Verminderung von bitteren und adstringenten Eigenschaften. Dt. Weinbau-Jahrb. 61: 201-207

Vidal, S., Cartalade, D., Souquet, J.M., Fulcrand, H. and Cheynier, V. 2002: Changes in proanthocyanidin chain length in wine-like model solutions. J. Agric. Food Chem. 50: 2261-2266

Vidal, S., Francis, L., Noble, A., Kwiatkowski, M., Cheynier, V. and Waters, E. 2004: Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. Anal. Chim. Acta (513): 57-65

Waterhouse, A.L. 2002: Wine phenolics. Ann. N.Y. Acad. Sci. 957: 21-36

Weber, F., Greve, K., Durner, D., Fischer, U. and Winterhalter, P. 2013: Sensory and chemical characterization of phenolic polymers from red wine obtained by gel permeation chromatography. Amer. J. Enol. Vitic. 64: 15-25

Weiand, J. 2009: Ausbau von Spätburgunder: Was bewirken Gärtemperatur und oenologische Maßnahmen? Teil I+II. Dt. Weinmagazin (19): 25-29; (20): 9-12

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