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Polyphenolic composition of German white wines and its use for the identification of cultivar

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The polyphenolic composition of 177 white wines of five white Vitis vinifera cultivars was analysed by means of HPLC/DAD. The polyphenolic composition of white wines showed great variability and was dependent upon cultivar and technology. Tyrosol was the dominating phenol in 'Bacchus', 'Müller-Thurgau' and 'Silvaner'. In 'Red Traminer' the main phenolic was p-coumaric acid and in 'Rieslaner' it was 3-hydroxybenzoic acid. Using principal component analysis 75% of the dependency of the polyphenols of 'Müller-Thurgau' and 'Silvaner' could be explained by the first three principal components, a definite classification of the cultivars by their polyphenolic profile, however, could not be achieved. The ratio between caftaric and coutaric acid varied between 0.5 to 10.6 in the studied cultivars, but it is suggested that it cannot be used for the taxonomic identification since it is too much dependent on non-genetical factors. While in literature a distinctive ratio of caftaric to coutaric acid of 12.2 (± 1.9) is published for 'Riesling' wines from the Rheingau region we found a ratio of 8.3 (± 4.1). Probably, the influence of oenological technologies is too strong, to keep the ratio of two polyphenols constant.
Keywords: Vitis vinifera, polyphenol, white wine, principal component analysis, classification, HPLC

Polyphenolzusammensetzung deutscher Weißweine und deren Nutzen zur Identifizierung der Rebsorte. *177 Weißweine fünf verschiedener Vitis vinifera-Rebsorten wurden mittels HPLC/DAD auf ihre Polyphenolzusammensetzung untersucht. Diese ist stark von der verwendeten Rebsorte und der Herstellung abhängig. Tyrosol dominierte in 'Bacchus', 'Müller-Thurgau' und 'Silvaner', in 'Roter Traminer' die p-Coumarsäure und in 'Rieslaner' 3-Hydroxybenzoesäure. Mittels einer Hauptkomponentenanalyse, die bei standardisierten Datensätzen von 'Müller-Thurgau' und 'Silvaner' durchgeführt wurde, konnten zwar die Abhängigkeiten der Polyphenole untereinander anhand der ersten drei Hauptkomponenten zu 75% erklärt werden. Eine definitive Aussage über die Zugehörigkeit einer Rebsorte anhand ihres Polyphenolprofils ist damit jedoch nicht möglich. Das Verhältnis von Caftar- zu Coutarsäure schwankte zwischen 0,5 und 10,6, es kann aber zur taxonomischen Unterscheidung der Rebsorten nicht herangezogen werden, da es zu sehr von nichtgenetischen Faktoren abhängt. Auch das von manchen Autoren in Rheingauer 'Riesling'-Weinen gefundene Verhältnis dieser beiden Substanzen zueinander von 12,2 (± 1,9) konnte nicht bestätigt werden. In eigenen Untersuchungen lag das Verhältnis weit tiefer (8,3) und auch die Standardabweichung war mit ± 4,1 weit größer. Hier machen sich oenologische Einflüsse vermutlich zu stark bemerkbar, als dass ein bestimmtes Verhältnis zweier einzelner Polyphenole zueinander über den Vinifikationsprozess hinaus erhalten bleiben könnte.*
Schlagwörter: Vitis vinifera, Polyphenol, Weißwein, Hauptkomponentenanalyse, Klassifizierung, HPLC

La composition polyphénolique des vins blancs allemands et son utilité pour l'identification du cépage. *La composition polyphénolique de 177 vins blancs de cinq différents cépages* Vitis vinifera *a été analysée par HPLC/DAD.*

Cette composition est fortement dépendante du cépage utilisé et du mode de production. Le tyrosol dominait dans 'Bacchus', 'Müller-Thurgau' et 'Silvaner', pour le 'Roter Traminer' c'était l'acide p-coumarique et pour le 'Rieslaner' l'acide 3-hydroxy-benzoýque. Il est vrai que les interdépendances des polyphénols ont pu être expliquées à 75 % au moyen de l'analyse en composantes principales, en utilisant les trois premières composantes principales, mais cela ne permet pas de faire une déclaration définitive relative à l'appartenance d'un cépage sur la base de son profil polyphénolique. Le rapport entre l'acide caftarique et l'acide coutarique variait entre 0,5 et 10,6. Il ne peut cependant pas être utilisé pour une distinction taxonomique des cépages, étant donné que la dépendance des facteurs non-génétiques est trop grande. Il n'a également pas été possible de confirmer le rapport entre ces deux substances de 12,2 (± 1,9), trouvé par quelques auteurs dans les vins 'Riesling' de la région allemande «Rheingau». Dans nos propres analyses, nous avons trouvé un rapport beaucoup plus bas (8,3) et la déviation standard de ± 4,1 était largement plus importante. Ici, les influences œnologiques sont probablement trop fortes pour que le rapport de deux polyphénols différents puisse se conserver au-delà du processus de vinification.
Mots clés : *Vitis vinifera*, polyphénols, vin blanc, analyse en composantes principales, classification, HPLC

A reliable method for the determination of the varietal origin of wines is of great interest to both wine industry and consumer. The individual polyphenolic fingerprint as reflected in the composition of hydroxycinnamic and hydroxybenzoic acids, as well as flavonoids and their derivatives, is distinctive for any plant. For red cultivars the analysis of the anthocyanin composition and the content of shikimic acid have been mainly used for distinguishing among red grape cultivars. Commonly, HPLC methods are used to analyse this part of the phenolic fingerprint. They have been successfully used in Germany for the authenticity control of 'Pinot noir' wines (Eder at al., 1994; Holbach et al., 1997; Holbach et al., 1998; Eder and Holbach, 1998). Although the polyphenolic composition of wine may be influenced by different factors such as varietal (Vrhovsek et al., 1997) and viticultural influence (Otreba et al., 2006), vinification, maturation and aging, the differences in the overall phenolic fingerprints might still be characteristic for each cultivar. Even in very old white wines, polyphenols can be detected as was shown for 'Riesling' wines from vintages 1892 to 1921 (Dietrich et al., 2004). In fact, in earlier studies HPLC methods have already been used for the identification of white grape cultivars. Ritter et al. (1994) showed that 'Riesling' wines from the German Rheingau region have a distinctive ratio of 12.2 (± 1.9) for the two polyphenols caftaric and coutaric acid. Presa-Owens et al. (1995) found a similar ratio for these two compounds in Spanish white wines from the Penedes region. They also showed that principal component analysis (PCA) of the polyphenolic fraction achieved a separation according to cultivar. In more recent studies polyphenolic fingerprints have been used to identify the vintage, geographic and even winery origin of red and white wines (Pour Nikfardjam et al., 2006; Peña-Neira et al., 2000; Tinttunen and Lehtonen, 2001; De Villiers et al., 2005). The current knowledge on the presence of phenolic compounds in wine can be found in the review of Monagas et al. (2005).

From our point of view it was interesting to see if various winemaking practices have a significant effect on the polyphenolic composition of German white wine. Importantly, we wanted to observe if the ratio of certain compounds remains constant regardless to winemaking practice and, therefore, can be used for the identification of the cultivar. Treutter (1989), Boursiquot (1987) and Ritter et al. (1994) stated that especially the ratio of caftaric and coutaric acid in grapes is genetically controlled, which we also wanted to verify within the scope of this study. Furthermore, as Ritter et al. (1994) described, the ratio being dependent on 'Riesling' from the Rheingau region only, we also collected samples from this region and analysed their polyphenolic composition.

Materials and Methods

Wine samples

177 white wines of five different *Vitis vinifera* cultivars ('Bacchus', 'Müller-Thurgau', 'Rieslaner', 'Silvaner', and 'Traminer') and different vinification techniques from vintages between 1989 and 1998 were collected from the Bayrische Landesanstalt für Weinbau und Gartenbau in Veitshöchheim (Germany). Seventeen 'Riesling' wines from the German Rheingau region were purchased from local wineries.

HPLC analysis

Analysis was performed using the method published by Rechner et al. (1998). All samples were directly injec-

ted into a HPLC (L-6200A, Merck-Hitachi) equipped with an autosampler (AS-2000, Merck-Hitachi), and a diode-array detector (DAD, LaChrom L-7210, Merck). Chromatograms were recorded at 280 and 320 nm. The column temperature was maintained at 25 °C with a Column Oven (Biorad). The injection volume was 10 µl. Identification and quantification of the compounds was carried out using DAD spectra. Hydroxycinnamic esters were quantified as their corresponding free acids.

Data analysis

All data analysis was carried out using Unscrambler Software (Camo, Norway, Version 7.6).

Total polyphenol content (Folin-Ciocalteu)

The total polyphenol content was determined using the method of Ritter (1994) with (+)-catechin as standard.

Results and discussion

General polyphenolic composition

Table 1 shows the mean polyphenolic composition of the 177 wines from Veitshöchheim analysed with HPLC.

The most abundant polyphenols in 'Bacchus' were tyrosol and coumaric acid followed by *p*-coumaroylglucosyltartrate (*p*-CGT) and protocatechuic acid. No flavan-3-ols could be detected in this cultivar. Generally speaking, 'Bacchus' had relatively low total concentrations of polyphenols (Folin: mean value 192mg/l). Also the concentration of cinnamic acid derivatives was quite low.

Tyrosol was also the most abundant phenolic compound in 'Müller-Thurgau'. Some wines also contained considerable amounts of catechin (7.0 mg/l), procyanidin B_2 (39.5 mg/l), epicatechin (12.3 mg/l), and p-coumaric acid (15.4 mg/l). This cultivar showed the highest amounts of tyrosol, procyanidin B_2, and epicatechin within the scope of this study. The low amounts of caftaric acid are probably due to enzymatic or oxidative losses during vinification.

We also analysed three 'Müller-Thurgau' wines, which had been fermented on the skins (not included in Table 1). These wines had especially high amounts of caftaric acid, catechin, GRP, procyanidin B_2, epicatechin, and rutin (quercetin-3-rutinosid) (Table 2). They also showed very high Folin values. The flavan-3-ols contributed the main part to the total polyphenol content. Interestingly, caftaric acid was then the most abundant polyphenol, but not tyrosol. This could be due to the fact that a high amount of flavan-3-ols leads to a strong antioxidative protection of oxidative sensitive phenols, such as hydroxycinnamic acids, and at the same time inhibits the oxidative formation of tyrosol out of the amino acid tyrosine.

'Rieslaner' showed to be the cultivar with the highest amount of polyphenols determined by HPLC. It was nearly twice as high compared to all the other cultivars analysed. This is mainly due to its high amounts of 3-hydroxybenzoic acid (Table 1). This cultivar also showed relatively high amounts of flavan-3-ols (16.3 mg/l for procyanidin B_2) and a relatively high mean content of caftaric acid (19.9mg/l). The high polyphenolic content could have provided a strong antioxidative capacity, which prevented caftaric acid from becoming oxidized. Because of the small number of samples (n = 7) it could not be determined if this is a characteristic for this cultivar.

Tyrosol was the dominant polyphenol in the 17 analysed 'Riesling' wines. The second most important polyphenol was caftaric acid. The wines showed generally low concentrations on flavan-3-ols, such as catechin, epicatechin, and procyanidin B_2, which could be an indicator for a gentle winemaking procedure. Also the levels on GRP were relatively low (mean: 1,7mg/l), which would support the aforementioned supposition that these wines were made using gentle processing regimes.

As expected, tyrosol was the most abundant polyphenol in 'Silvaner' followed by caftaric acid. This cultivar also had high amounts of flavan-3-ols, such as catechin and epicatechin. This could be due to longer skin contact or harsher pressing during winemaking. These factors are known as leading to better extraction of these compounds out of the grape seeds.

Interestingly, *p*-coumaric acid was the polyphenol with the highest concentration in 'Red Traminer' followed by tyrosol, caffeic acid and *p*-CGT. 'Red Traminer' is known to produce small amounts of anthocyanins at a very ripe stage, as the name indicates. In red grapes *p*-coumaric acid is produced to transfer the anthocyanin in the acylated form into the cell vacuole during pigment accumulation. This could eventually explain the high coumaric acid amounts in this cultivar. Caftaric acid concentrations were below 10 mg/l and no flavan-3-ols could be found in any of the wines; again, probably due to oxidative processes or gentle whole bunch pressing (no or only little extraction of flavan-3-ols out of the seeds).

Table 1: Mean (Min-Max) polyphenolic composition (mg/l) of German wines (Vintage 1989 - 1998)

Cultivar	Bacchus	Müller-Thurgau	Rieslaner	Riesling	Silvaner	Traminer
Number of Samples	N = 14	N = 75	N = 7	N = 17	N = 68	N = 10
Vintage	1997	1989-98	1992-96	1996-98	1993-98	1998
Gallic acid	0,8 (n.q.-2,9)	1,3 (n.q.-10,1)	0,6 (n.q.-1,7)	n.q.	1,6 (n.q.-14,8)	1,4 (n.q.-2,8)
Protocatechuic acid	4,5 (1,7-11,0)	2,1 (n.q.-7,7)	2,4 (0,7-3,7)	n.q.	3,5 (n.q.-11,0)	3,0 (1,8-4,4)
Tyrosol	13,9 (9,9-22,0)	19,9 (n.q.-39,2)	16,1 (10,8-19,6)	18,0 (n.q.-42,2)	17,4 (3,5-26,7)	16,0 (7,5-24,1)
3-Hydroxybenzoic acid	n.d.	0,2 (n.q.- 12,0)	23,1 (n.q.-36,8)	n.q.	0,9 (n.q.-38,3)	n.d.
Caftaric acid	1,4 (n.q.-3,6)	4,0 (n.q.-19,9)	19,9 (17,2-28,4)	13,7 (0,5-25,9)	8,3 (n.q.-40,2)	2,8 (n.q.-21,1)
Catechin	n.d.	0,3 (n.q.-7,0)	6,0 (n.q.-10,9)	n.q.	1,1 (n.q.-26,3)	n.d.
GRP	2,3 (n.q.-12,4)	3,7 (n.q.-18,6)	8,1 (6,6-12,8)	1,7 (n.q.-5,1)	4,1 (n.q.-18,0)	4,0 (1,5-8,4)
Procyanidin B2	n.d.	1,7 (n.q.-39,5)	8,1 (n.q.-16,3)	n.q.	0,3 (n.q.-6,7)	n.d.
p-CGT	8,2 (n.q.-16,7)	4,3 (n.q.-23,0)	2,1 (n.q.-3,9)	0,5 (n.q.-1,7)	10,6 (n.q.-61,4)	7,5 (n.q.-24,2)
Syringic acid	n.d.	n.d.	0,6 (n.q.-2,1)	n.q.	n.d.	n.d.
Caffeic acid	1,9 (n.q.-10,0)	0,7 (n.q.-2,5)	2,4 (1,1-2,9)	1,6 (n.q.-3,9)	2,0 (n.q.-13,5)	8,4 (2,1-25,9)
Epicatechin	n.d.	0,3 (n.q.-12,3)	3,3 (n.q.-5,7)	0,9 (n.q.-6,4)	0,3 (n.q.-10,3)	n.d.
Coutaric acid	2,5 (n.q.-6,3)	2,0 (n.q.-8,4)	0,6 (n.q.-2,9)	1,7 (0,7-3,0)	2,7 (n.q.-6,9)	1,3 (n.q.-7,5)
Sinapinic acid	n.d.	0,1 (n.q.-3,0)	1,0 (n.q.-5,1)	n.q.	0,2 (n.q.-4,7)	n.d.
Fertaric acid	1,0 (n.q.-2,5)	1,3 (n.q.-2,7)	3,6 (2,5-4,8)	1,1 (n.q.-2,6)	1,6 (n.q.-3,3)	2,6 (n.q.-5,8)
p-Coumaric acid	12,4 (6,2-18,8)	4,4 (n.q.-15,4)	4,5 (2,2-5,7)	1,3 (n.q.-3,7)	8,2 (0,8-33,3)	21,9 (8,5-47,2)
Ferulic acid	1,1 (n.q.-3,5)	0,3 (n.q.-1,7)	3,2 (1,0-4,6)	0,7 (n.q.-4,7)	0,6 (n.q.-5,5)	1,3 (n.q.-3,6)
Ellagic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin-3-galactosid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Quercetin-3-rutinosid	n.d.	0,1 (n.q.-3,5)	n.d.	n.d.	n.d.	n.d.
Quercetin-3-glucosid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
trans-Resveratrol	n.d.	0,2 (n.q.-1,5)	n.d.	n.q.	0,1 (n.q.-1,7)	n.d.
Quercetin	n.d.	n.d.	1,4 (n.q.-4,1)	n.q.	n.d.	n.d.
Sum (HPLC)	*50 (37-65)*	*49 (24-97)*	*111 (55-140)*	*48 (14-87)*	*64 (22-124)*	*70 (29-137)*
Folin (mg/l)	*192 (131-305)*	*224 (96-379)*	*287 (248-325)*	*257 (178-376)*	*261 (131- 509)*	*264 (166-356)*

n.d. = not detected, n.q. = not quantified

In contrast to the results for 'Riesling' wines published by Ritter et al. (1994), caftaric acid was not the dominant compound in all wines. Tyrosol dominated in 'Müller-Thurgau' and 'Silvaner', 3-hydroxybenzoic acid in 'Rieslaner' and *p*-coumaric acid in 'Traminer', respectively. These compounds are indicators for a relatively oxidative handling of the grape material; e.g. tyrosol is derived from the amino acid tyrosine through oxidative and enzymatic processes during vinification (Ritter, 1994).

The ratio between caftaric and coutaric acid showed large differences between different cultivars as well as

Table 2: Polyphenols (mg/l) in three 'Müller-Thurgau' wines fermented on the skins

	Wine 1	Wine 2	Wine 3
Gallic acid	7.8	8.6	32.0
Tyrosol	36.7	40.7	31.2
Caftaric acid	61.3	57.6	92.6
Catechin	53.0	1.8	102.0
GRP	n.d.	40.0	n.d.
Procyanidin B2	37.6	53.7	65.1
Caffeic acid	3.5	6.3	7.4
Epicatechin	21.6	53.3	25.3
Quercetin-3-rutinosid	6.4	5.9	7.5
Folin	1005	755	1283

within a single cultivar (Table 3). Compared to the results published by RITTER et al. (1994) each cultivar has a significant different caftaric/coutaric acid ratio. But even within a cultivar there are also large differences. 'Silvaner' has a mean caftaric/coutaric acid ratio of 4.3 with a large span between 0.0 and 18.5 and, thus, the standard deviation is also very high, 4.99. Furthermore, some cultivars show nearly the same mean and min/max values (e.g. 'Müller-Thurgau' and 'Silvaner', ref. Table 3), that could easily lead to misinterpretations and false classification of the cultivar.

According to RITTER et al. (1994) the distinctive ratio of 12:1 between caftaric and coutaric acid is only valid for 'Riesling' wines of the German 'Rheingau' region. Thus, seventeen 'Riesling' wines had been purchased in local stores and also analysed on their polyphenolic content. In contrary we found a mean ratio of 8.3 with a span between 0.7 and 21.0. Unfortunately, RITTER et al. (1994) did not state their minimum and maximum ratios observed during their studies making it impossible to compare the accuracy of both analytical data sets.

Table 3: Caftaric / coutaric acid ration in 177 German white wines

Caftaric / coutaric acid ratio	Mean	Min	Max	Median	STDev
Bacchus 1997 (n = 14)	0,5	0,0	0,7	0,6	0,21
Müller-Thurgau 1989-98 (n = 78)	3,8	0,0	15,9	1,1	4,18
Rieslaner 1992-96 (n = 7)	10,6	9,8	11,5	10,6	1,18
Silvaner 1993-98 (n = 68)	4,3	0,0	18,5	1,1	4,99
Roter Traminer 1998 (n = 10)	0,9	0,0	2,8	0,0	1,63
Riesling 1991/92 (n = 53)*	12,0	n.a.	n.a.	n.a.	1,9
Riesling, own results (n = 17)	8,3	0,7	21,0	8,1	4,1

*RITTER et al., 1994

Principal Component Analysis (PCA)

PCA was applied on phenolic data of 'Müller-Thurgau' and 'Silvaner' to determine dependencies of certain polyphenols. PCA was only carried out with the data sets that showed a normal distribution like caftaric, ferulic, caffeic and protocatechuic acid. If a data set like fertaric acid and tyrosol did not show a normal distribution the logarithmic data were used. All other polyphenols were not included in the PCA. Data sets were standardised before PCA calculation ($x_{in} = x_i$ / STDev) and wines fermented on the skins were not included in the PCA. Figure 1 shows the results of the PCA with seven polyphenols (gallic, caftaric, ferulic, caffeic and protocatechuic acid; logarithmically transformed: fertaric acid, and tyrosol).

Both cultivars can be separated into two 'clouds'. The so-called 'x-loadings' (arrows) show the dependencies of each polyphenol and their influence on the respective cultivar (cloud). If the arrows are right-angled there is no correlation between those two polyphenols. If they point into the same or into the opposite direction there is a positive or a negative correlation, respectively, between those polyphenols. Between tyrosol and ferulic and protocatechuic acid, respectively, a slightly negative correlation could be found. Oxidative processes are known to lead to higher concentrations in tyrosol (RITTER, 1994) and decrease the amounts of hydroxycinnamic acids. Protocatechuic and ferulic acid, and fertaric, caftaric and caffeic acid show a positive correlation. This means that with higher contents of ferulic acid there was also a higher amount of protocatechuic acid in the wine.

Because of their larger distance from the point of origin protocatechuic and ferulic acid and tyrosol had the largest influence on both cultivars (with consideration of the data transformation and standardisation undertaken in this special statistical evaluation). The explained variance of all cases and their dependencies, respectively, can, thus, be explained up to 75% by the first three principal components (data not shown). Protocatechuic and ferulic acids as representatives of the hydroxybenzoic and hydroxycinnamic acids, respectively, have by any means a far stronger influence on the polyphenolic composition of 'Müller-Thurgau' and 'Silvaner' than caftaric acid, although the latter is referred to as the most abundant and important polyphenol in German white wines in literature (RITTER et al., 1994).

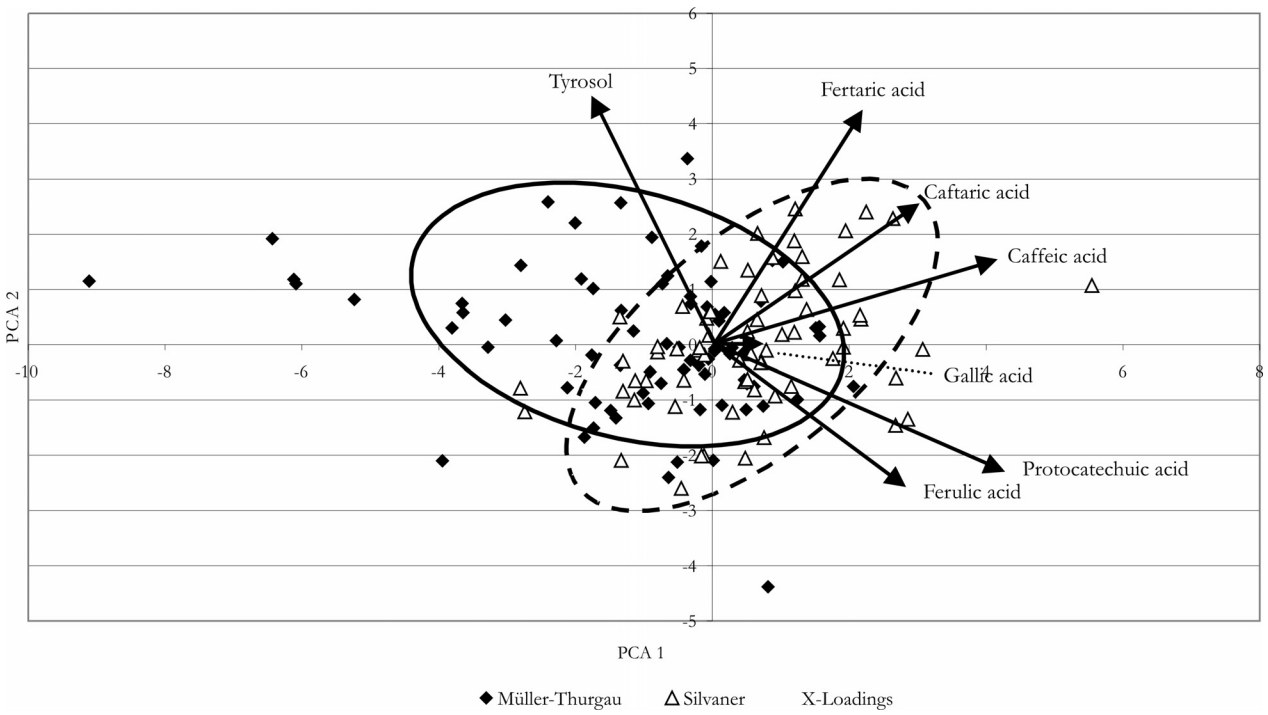

Figure 1: PCA of 'Müller-Thurgau' and 'Silvaner' for 7 polyphenols

This shows, in accordance with RITTER (1994) and POUR NIKFARDJAM (2001), that caftaric acid is very prone to oxidative processes and is probably lost intensively through enzymatic or chemical reactions with glutathion to GRP during vinification. Also the oxidatively derived polyphenol tyrosol has a strong influence on the polyphenolic fingerprint. In fact, tyrosol is often much more dominant in German wines than caftaric acid and indicates the relatively oxidative handling of grapes and wine (POUR NIKFARDJAM, 2001) thereby supporting the assumption that hydroxybenzoic and hydroxycinnamic acid concentrations decrease during vinification - mainly through oxidation and fining.

Although PCA is a good statistical method to explain dependencies between polyphenolic compounds in the two white cultivars 'Müller-Thurgau' and 'Silvaner', it cannot be used to distinguish between cultivars according to their polyphenolic composition. Varietal as well as oenological and seasonal influences seem to affect the polyphenolic composition of white wines too strongly. Therefore, the ratio between two polyphenols cannot be expected to remain constant as known for red cultivars and their anthocyanin composition (EDER et al., 1996).

So these results are in opposition to the data recently published by DE VILLIERS et al. (2005). According to their results, 97.4% of the wines made from white grape cultivars could be correctly classified on basis of their polyphenolic profile. Other recently published methods, such as FTIR techniques, have also been successfully used for the discrimination of red wine cultivars (EDELMANN et al., 2001; PICQUE et al., 2002). Therefore, research should focus on these techniques and test their possible application for the identification of white wine cultivars.

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