EVALUATION OF VOLATILE COMPOUNDS DURING THE RIPENING IN SOUTH MORAVIAN 'GEWÜRZTRAMINER' AND 'SAUVIGNON BLANC' FROM THE PÁLAVA REGION

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The volatile compounds present in the grape varieties 'Gewürztraminer' and 'Sauvignon Blanc' were examined at three different stages of ripening. The formation of volatile compounds in grapes is a dynamic process, and generally they are present in only small quantities or even completely absent at harvest time. However, these compounds are significant in providing a possible means to identify specific grape varieties, or at least distinguish one variety from another. Four volatiles were found to be important for differentiating between the different ripening stages: damascenone, (Z)-hex-3-en-1-ol, nonanolactone and 3-methyl-1-pentanol. Two significant volatiles, useful for differentiating the two grape varieties by log regression analysis, are butyl acetate and 3-methyl butan-1-ol. Terpenes (limonene, cis-geraniol and terpineol) with their floral and fruity notes are important compounds for the varietal aromas when present at sufficiently high concentrations, but are present at all stages of ripening and their concentrations start to decrease at the overripe stage. The first two principal components, PC1 and PC2 for ripe fruit, accounted for 78.6 % of the total variability (contributing 43.7 % and 34.9 %, respectively).
Keywords: grape varieties, volatiles, ripening, 'Gewürztraminer', 'Sauvignon Blanc'

Untersuchung flüchtiger Verbindungen während der Reife in 'Gewürztraminer' und 'Sauvignon Blanc' aus der Pálava-Region in Südmähren. Die flüchtigen Verbindungen in den Rebsorten 'Gewürztraminer' und 'Sauvignon Blanc' wurden zu drei verschiedenen Reifestadien untersucht. Die Bildung dieser Verbindungen in Trauben ist ein dynamischer Prozess, und im Allgemeinen sind sie zur Erntezeit nur in geringen Mengen oder gar nicht vorhanden. Aber diese Verbindungen sind wichtig für die Identifizierung spezifischer Rebsorten oder zumindest dafür, eine Sorte von einer anderen zu unterscheiden. Vier flüchtige Bestandteile erwiesen sich als wichtig zur Differenzierung verschiedener Reifestadien: Damascenon, (Z)-Hex-3-en-1-ol, Nonanolacton und 3-Methyl-1-pentanol. Zwei wesentliche flüchtige Verbindungen für die Unterscheidung der beiden Sorten mittels Logregressionsanalyse sind Butylacetat und 3-Methyl-butan-1-ol. Terpene (Limonen, cis-Geraniol und Terpineol) mit ihren blumigen und fruchtigen Noten sind – in ausreichend hohen Konzentrationen – wichtige Verbindungen für die sortentypischen Aromen. Sie sind in allen Reifestadien vorhanden, nehmen aber im Stadium der Vollreife ab. Die ersten zwei Hauptkomponenten, PC1 und PC2 für reife Trauben, waren für 78,6 % der Gesamtvariabilität (43,7 % bzw. 34,9 % verantwortlich.
Schlagwörter: Rebsorten, flüchtige Verbindungen, Reife, 'Gewürztraminer', 'Sauvignon Blanc'

Grape ripening is a physiological period that starts at the moment of veraison and lasts until the fruit is fully ripe. The concentration of varietal aroma compounds in grapes is influenced by several factors, such as grape variety and degree of maturity, vintage, climate and vineyard management techniques (Bueno et al., 2003; Oliveira et al., 2006; Vilanova et al., 2007). C6-aldehydes and alcohols are formed from linoleic acid and linolenic acid when grapes come into contact with air, and are formed by the actions of lipoxygenase, peroxidase and alcohol dehydrogenase enzymes. Terpenes are important compounds contributing to the varietal aroma with their floral and fruity notes, but are present in green berries in only very small amounts. Their concentrations gradually increase during ripening until around maturity, after which concentrations decrease (Wilson et al., 1984; Günata et al., 1985). The aim of this work was to study the aroma profile of two grape varieties using SPME coupled with GC-MS and then use principal component analysis (PCA) at each stage of ripening to identify the key compounds.

MATERIALS AND METHODS

Both varieties were grown in the same area (Pavlov, South Moravia). Berries were harvested and transported with minimal delay (approx. 2 hrs) to the Postharvest Laboratory in Lednice, where they were juiced and frozen at -22 °C. Prior to analysis the samples were thawed and 2 g samples of juice were weighed into vials. A manual solid phase microextraction (SPME) fibre holder coated with a 100 μm layer of poly-dimethylsiloxane (PDMS), purchased from Supelco (Bellefonte,USA), was chosen to absorb the volatile components of the fruit samples. First, the extraction head of the SPME was conditioned in the sample valve of the GC-MS at a temperature of 250 °C for 5 min, and then the needle of the SPME device pierced the septum of the vial and the fiber was exposed for 30 min to the headspace of the vial at 250 °C. Subsequently, the needle was removed from the sample vial. Finally, the needle was manually inserted into the gas chromatography injector, where the samples were thermally desorbed and analyzed. The desorption time was 5 min at 250 °C. GC-MS measurements were made using a gas chromatograph (7890A; Agilent Technologies, Inc., Santa Clara, USA) interfaced to a quadrupole mass spectrometer (Agilent GC MSD 597), using the NIST 98 library of mass spectra. Analyses were separated using a DW WAX fused silica capillary column of 30 m × 0.25 mm with a 0.25 μm film, (Agilent Technologies, USA), which was inserted directly into the ion source of the MS. Compounds were provisionally identified using the NIST mass spectra library search, and the identity of most of these compounds was then confirmed by comparing their mass spectra and retention times with those obtained for a standard.

All statistical analyses were performed using the SAS statistical software package version 9.2. (SAS Institute Inc., Cary, USA). For each grape variety, descriptive statistics (mean ± standard error) at each stage of maturity were calculated for each parameter under investigation. A stepwise log regression model was used to explore the effect of all parameters of the volatiles as explanatory variables in a simple binary response (ripe/overripe). A binary value of 0 corresponds in this model to the ripe stage and the value 1 codes for the overripe stage. Principal component analysis (PCA) was used to discriminate the varieties at each of the two stages of ripeness. The first two principal compounds were chosen as a linear combination of the observed variables, selected in such a way that the resulting compounds accounted for the maximum amount of variation in the set of volatile and non-volatile variables. Before proceeding with the statistical analysis the data matrix was standardized by setting mean values at zero.

RESULTS AND DISCUSSION

LEVELS OF VOLATILES DURING RIPENING

Veraison is an important etiological stage which visibly marks the onset of ripening, and many changes in berry development begin at this time. Nineteen alcohols were detected in this study, and all were detected before veraison in both varieties (ethanol, n-butanol, 2-methylbutanol, 3-methylbutanol, 2-methyl pentanol, 3-methyl pentanol, 4-methyl pentanol, n-pentanol, n-hexanol, 2-furfuryl alcohol, 2-heptanol, ethylhexanol, (Z)-3-octenol, (Z)-3-hexenol, (E)-2-hexenol, 2-octanol, fenol, 3-methyl-2-butenol and benzyl alcohol). In most cases their concentrations decreased steadily during ripening, but ethanol and benzyl alcohol tended to increase, especially in the berries of 'Sauvignon Blanc'. Five C6-compounds, hexanal, (E)-2-hexenol-1, 1-hexanol, (Z)-3-hexenal, and (E)-2-hexenol were found in both varieties. The two dominant volatiles here were hexanal (with levels in the 'Gewürztraminer' and 'Sauvignon Blanc' of 297.0 ± 86.5 µg/kg and 502.9 ± 84.6 µg/kg respectively) and (E)-2-hexenol (with levels in the 'Gewürztraminer' and 'Sauvignon Blanc' of 2144 ± 323 µg/kg and 3003 ±264 µg/kg, respectively). These C6-compounds showed similar trends in both varieties: increasing during the early stages, then decreasing, but the stage, at which they attained peak production, varied. Fifteen carbonyls were detected, in both varieties: (2-methylbutanal, 3-methylbutanal, n-hexanal, (E)-2-hexenal, (Z)-3-hexenal, heptanal, n-octanal, (E)-2-octenal, n-nonanal, (E)-2-nonenal, 2-(E)-6-(Z)- nonadienal, n-decanal, (E)-2-decenal, phenylacetaldehyde and 5-methyl-2-furfural), while there was only one ketone (acetophenone). The concentrations of the twelve esters detected, typically ranged from just 0.1 µg/kg (ethyl undecanoate) to 5.000 µg/kg (3-methylbutyl pentanoate), but the most abundant ester by far was butyl acetate in the 'Gewürztraminer' with 469.5 ±131.8 µg/kg. Butyl 2-methyl butyrate was present in significant, but almost identical amounts in both varieties, but steadily decreased during ripening.

TERPENES IN BOTH VARIETIES

Terpenes are mainly responsible for the flavour attributes typical for 'Gewürztraminer' (Marais, 1987; Girard et al., 2002). Our study identified a total of 9 terpenes, of which limonene was the most abundant, ranging from 226.8 ± 4.8 µg/kg to 277.1 ± 59.7 µg/kg in 'Gewürztraminer' and decreasing in 'Sauvignon Blanc' from 252.3 ± 26.3 µg/kg to 86.07 ± 9.03 µg/kg. Regarding 'Gewürztraminer' grapes, certain volatile constituents provide the characteristic spicy and floral flavour of the finished wine. Maximizing the development of these desirable quality characteristics during ripening is an important objective for both growers and winemakers. Terpenes, as well as other grape-derived flavour compounds, are present as free volatiles and as sugar-bound precursors (Hardy, 1970;Wilson et al., 1986). In terms of terpenes (Z)-geraniol ranked highest in concentration (63.0 to 67.5 µg/kg, followed by α-terpineol (17.1 to 19.1 µg/kg, (R)-(+)-ß-citronellol (0.30 to 1.06 µg/kg) and ß-ionone (3.0 to 4.1 µg/kg). Most of the volatile compounds found in 'Gewürztraminer' and other grape varieties have already been reported (Gómez et al., 1995; Marais, 1987; Williams et al., 1982). The norisoprenoids are generally considered to be derived from carotenoids by oxidative degradation (et al., 2002). Several terpenoids, including (Z)-linalol oxide, ß-damascenone, linalool and geraniol, are key odour-active compounds responsible for the overall flavour profile of 'Gewürztraminer' wine (Guth, 1997; Onug and Acree, 1999). Lactone is a pleasant smelling compound found naturally in grapes (Guth, 1996; Pérez-Olivero et al., 2014). However, three more lactones, ϒ-caprolactone, ϒ-nonanolactone and ϒ-decalactone, were found in the two varieties examined in this study, with the highest concentrations for ϒ-decalactone (2.55 ± 0.07 µg/kg and 1.16 ± 0.15 µg/kg for 'Gewürztraminer' and 'Sauvignon Blanc', respectively). C13-norisoprenoids are usually more abundant in berries at maturity than was found in this study, for both varieties, where ß-ionone ('Gewürztraminer') and α-damascenone ('Gewürztraminer' and 'Sauvignon Blanc') were only found at levels of 22.36 ± 4.59 µg/kg and 28.29 ± 1.85 µg/kg, respectively. Baumes et al. (2002) have shown that these compounds are degradation products of carotenoids and Mendes-Pinto (2009) has also shown that norisoprenoids can be formed by the direct degradation of carotenoids such as ß-carotene and neoxanthin.

VOLATILE COMPOUNDS THAT CAN BE USED TO DIFFERENTIATE RIPENING STAGES AND VARIETIES

Aroma compounds were identified and quantified at three different stages of ripening: unripe, ripe and overripe. To examine the association between concentrations of volatile parameters in the grapes and the level of ripening (ripe vs. overripe) as a binomial response variable a stepwise log regression model was used. The aim was to identify the main volatiles associated with ripening. A log regression was also applied to determine which parameters might be useful in differentiating the two varieties. In this case, the response variable is categorical, using the varieties themselves (Table 1 and 2). Before making the calculations, all input independent variables were standardized by setting mean values to zero, in order to highlight the variability around their mean values. The significance of the estimated χ-square score analysis of compounds showed that there are four volatile compounds which were identified as being useful in differentiating the three ripening stages using log regression analysis: α-damascenone, (Z)-hex-3-en-1-ol, ϒ-nonanolactone and 3-methyl-1-pentanol (Table 1). Two volatile compounds were shown to be useful in distinguishing the two grape varieties, butyl acetate and 3-methyl-butan-1-ol, although the first is capable of much higher resolution values (Table 2).

Table 1: Significant volatile parameters useful for differentiating ripening stages, using log regression analysis

Parameter	DF	Score χ-square	Pr > χ-sqare
α-damascenone	1	14.3506	0.0002
(Z)-hex-3-en-1-ol	1	8.2166	0.0042
γ-nonanolactone	1	2.9702	0.0848
3-methyl-1-pentanol	1	0.2009	0.6540

Table 2: Significant volatile parameters useful for differentiating grape varieties, using log regression analysis

Parameter	DF	Score χ-square	Pr > χ-sqare
Butyl acetate	1	13.0501	0.0003
3-methyl butan-1-ol	1	0.5710	0.4499

Table 3: Eigenvectors of PCA components for differentiating grape varieties at each ripening stage

Parameter	Non-ripe grapes		Ripe grapes		Overripe grapes	
	COMP1	COMP2	COMP1	COMP2	COMP1	COMP2
α-damascenone	0.213	-0.261	0.071	-0.129	0.003	0.256
(Z)-hex-3-en-1-ol	0.407	-0.124	-0.090	-0.049	-0.089	-0.076
γ-nonanolactone	-0.222	-0.250	0.397	0.890	0.056	0.757
3-methyl-1-pentanol	0.422	0.813	0.740	-0.289	0.639	0.423
Butyl acetate	-0.743	0.414	0.474	-0.139	0.762	-0.420
3-methyl butan-1-ol	0.103	0.146	-0.238	0.292	-0.010	0.020

Table 4: Eigenvectors of PCA components for differentiating ripening stages in each grape variety

Parameter	Gewürztraminer		Sauvignon Blanc	
	COMP1	COMP2	COMP1	COMP2
α-damascenone	0.420	-0.078	0.509	0.119
(Z)-hex-3-en-1-ol	0.348	-0.037	0.535	0.501
γ-nonanolactone	-0.349	-0.091	-0.444	0.794
3-methyl-1-pentanol	-0.563	0.410	0.250	0.301
Butyl acetate	0.146	0.871	0.000	0.000
3-methyl butan-1-ol	0.493	0.238	0.442	-0.117

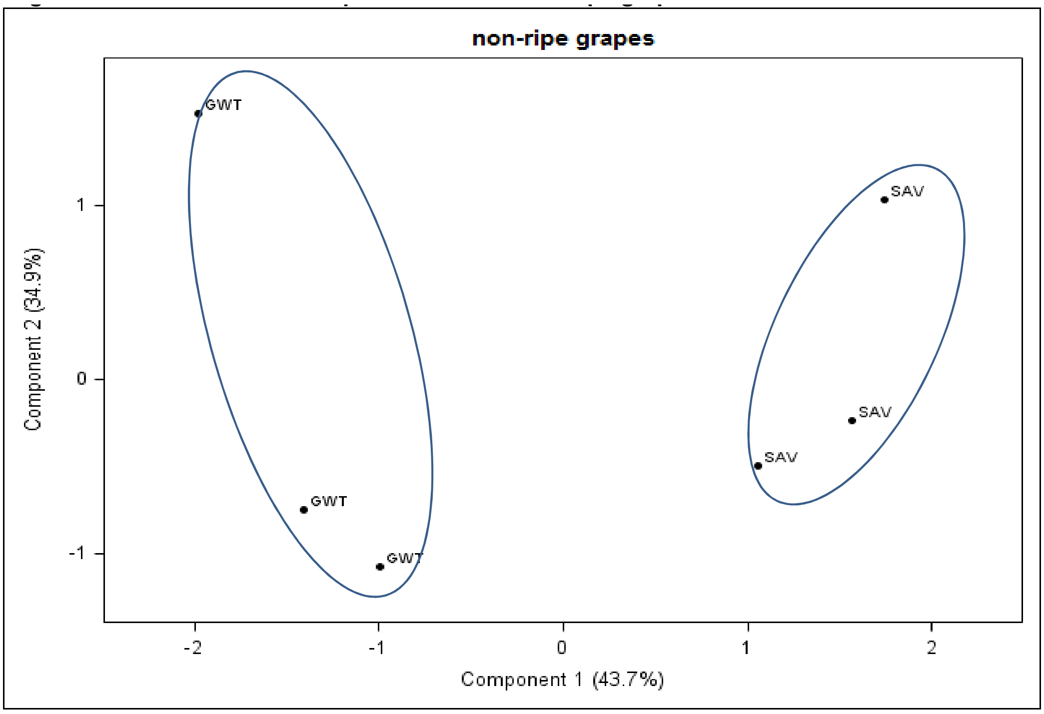

Fig. 1: Patterns of PCA component scores - non-ripe grapes

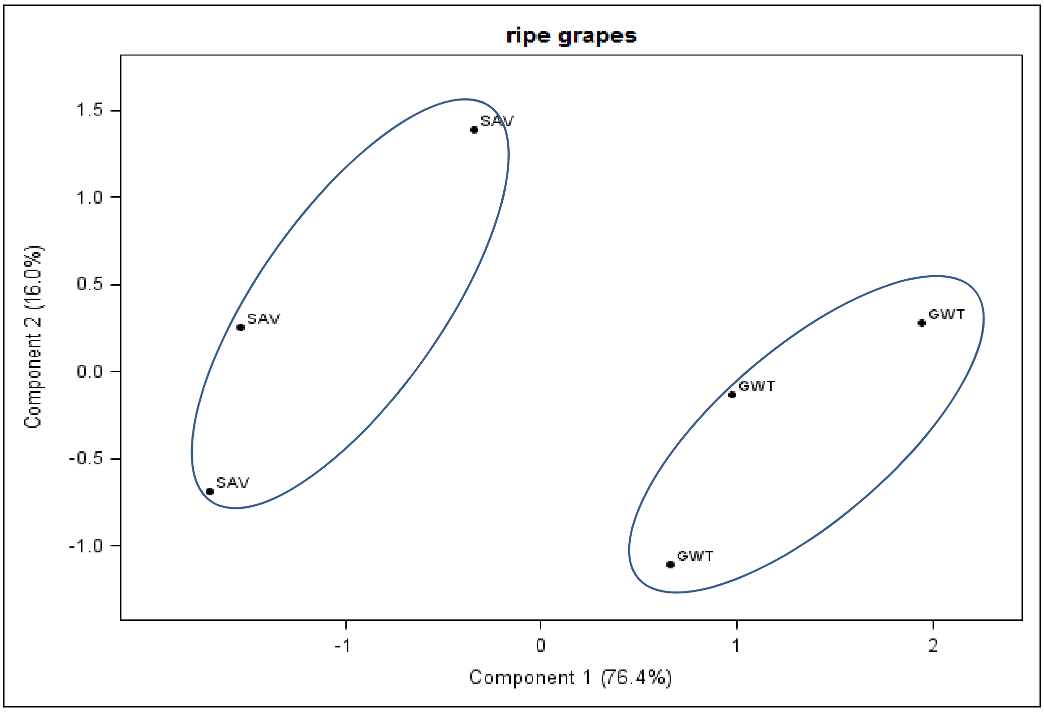

Fig. 2: Patterns of PCA component scores - ripe grapes

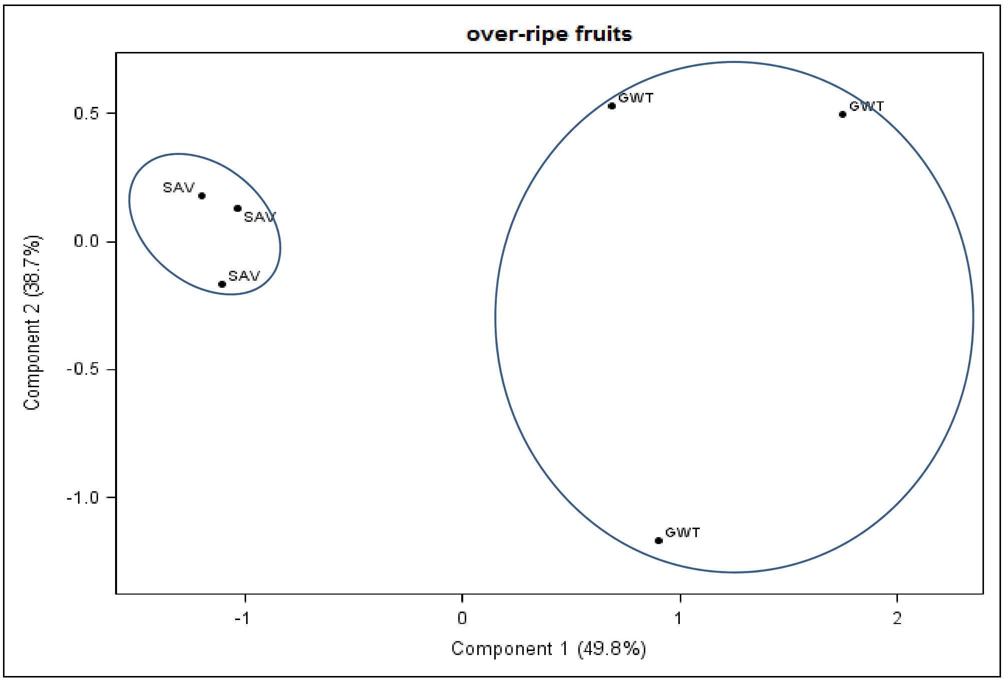

Fig. 3: Patterns of PCA component scores - overripe grapes

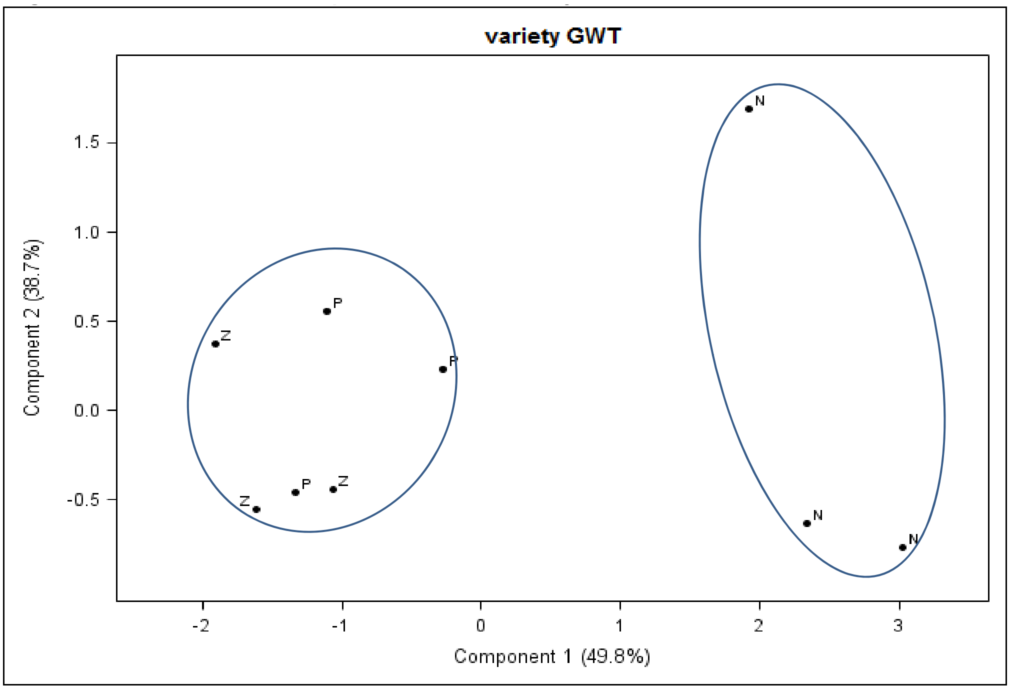

Fig. 4: Patterns of PCA component scores: 'Gewürztraminer'; N = non-ripe, Z = ripe, P = overripe grapes

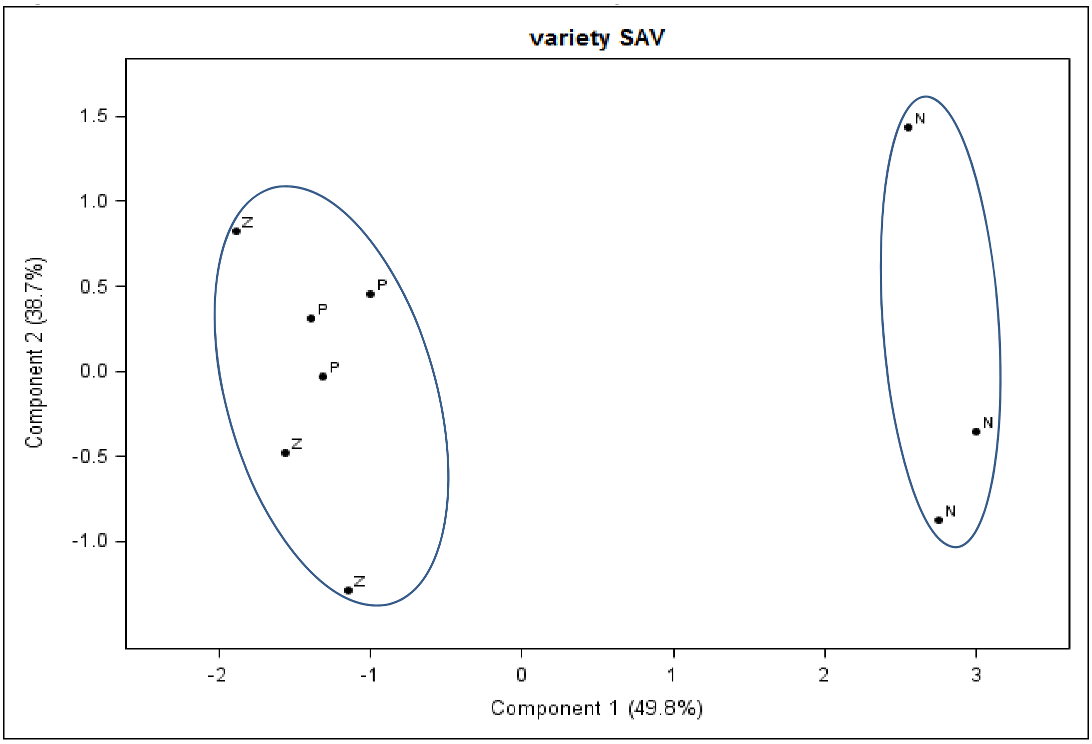

Fig. 5: Patterns of PCA component scores: 'Sauvignon Blanc'; N = non-ripe, Z = ripe, P = overripe grapes

PRINCIPAL COMPONENT ANALYSIS (PCA)

68 aroma compounds were chosen as the input parameters for PCA analysis. Six were found to be sufficient in order to distinguish the grape varieties at the earlier stage of ripeness (non-ripe to overripe): α-damascenone, (Z)-hex-3-en-1- ϒ-nonanolactone, 3-methyl-1-pentanol, butyl acetate and 3-methyl butan-1-ol (Table 3). For non-ripe fruit the first principal component (PC1) explained 43.7 % of the data variation and showed a high correlation with 3-methyl-1-pentanol (0.422), (Z)-hex-3-en-1-ol, (0.407), butyl acetate (-0.743), and also a low correlation with 3-methyl butan-1-ol. The second principal component (PC2) at the same stage of maturity explained 34.9 % of the data variation and correlated with 3-methyl-1-pentanol (0.813) and butyl acetate (0.414) (Table 3). Figure 1 shows the two varieties as defined by PC1 and PC2, which together explain 78.6 % of the data variation. As can be seen, PC1 shows a high positive value for the variety 'Gewürztraminer' and a somewhat less positive value for 'Sauvignon Blanc'. Therefore PC1 mainly shows a separation between the two varieties based on differences in the matrices of their volatile constituents. PC2, however, showed minimal differences between the two varieties. In Figures 2 and 3, that express the ripening stages as ripe and overripe, the two varieties are clearly distinguished, which proves that the compounds listed in Table 3 can perfectly well differentiate all three stages of ripening. The three stages of ripening are distinguished (Table 4) by just three compounds. Of these, butyl acetate, which had an average value in 'Gewürztraminer' of 469.5 ± 131.8 µg/kg, is useful because it is not found at all in 'Sauvignon Blanc'. The other two compounds, (Z)-hex-3-en-1-ol and ϒ-nonanolactone, have significantly different concentrations in 'Sauvignon Blanc' compared to 'Gewürztraminer'. In Figures 4 and 5, only the stage of non-ripe grapes for both varieties can be distinguished from the other two stages (ripe and overripe).

CONCLUSION

An HS-SPME-GC-MS method was used to determine the concentrations of 68 volatile compounds in two grape varieties ('Gewürztraminer' and 'Sauvignon Blanc') grown in the Pálava region of South Moravia. During ripening, substantial changes take place in the composition of the grapes. The concentrations of aroma compounds at different stages of ripeness were statistically analyzed using log regression analysis and PCA was successful in differentiating the two grape varieties at unripe, ripe and overripe stages of development. Although the numbers of volatile compounds in each sample were similar, the absolute amounts of total volatiles and those of certain individual compounds did vary between the varieties and also with the stages of ripening. The resolution of both varieties was demonstrated by means of two compounds, butyl acetate and 3-methyl-butan-1-ol, although the first mentioned has much higher resolution values.

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