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Analysis of 2-aminoacetophenone by direct-immersion solid-phase microextraction and gas chromatography–mass spectrometry and its sensory impact in Chardonnay and Pinot gris wines

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Abstract

A rapid immersion solid-phase microextraction (SPME) and gas chromatography (GC)–mass spectrometry (MS) method was developed to quantify 2-aminoacetophenone (2-AAP) in white wines. Thirteen white wines, including Pinot blanc, Pinot gris and Chardonnay were analyzed, and the concentrations of 2-AAP in these wines were ranging from <1 to 13 ng/L. Sensory properties of 2-AAP in Pinot gris and Chardonnay were studied using flash profiling method. Aroma descriptors such as fruity, floral, buttery, oaky/toasty, painty, shoe insole, mothball and medicinal were developed for 2-AAP. Sensory evaluation demonstrated that when wines were spiked with increasing concentrations of 2-AAP, wine aromas (i.e. fruity and floral) were gradually lost while 2-AAP associated aromas (i.e. painty, mothball and medicinal) appeared. The 2-AAP associated aromas became prominent when the concentration of 2-AAP was increased to 0.5 μg/L.

Keywords: 2-Aminoacetophenone; White wine; Off-flavour; UTA; SPME

1. Introduction

2-Aminoacetophenone (2-AAP) has been identified as an important aroma-active compounds in many food products (Buttery & Ling, 1994; Karagül-Yüceer, Drake, & Cadwallader, 2001; Karagül-Yüceer, Cadwallader, & Drake, 2002; Karagül-Yüceer, Vlahovich, Drake, & Cadwallader, 2003; Preininger & Ullrich, 2001) including wines (Culleré, Escudero, Cacho, & Ferreira, 2004; Ferreira, Ortín, Escudero, López, & Cacho, 2002; Rapp, Versini, & Ullemeyer, 1993). When its concentration in the wine exceeds its sensory threshold, 2-AAP causes an "atypical aging" or "untypical aging" (UTA) off-flavour (Rapp et al., 1993; Rapp, 1998; Sponholz & Hühn, 1996). Although the sensory description of UTA off-flavour varies significantly from one wine growing region to another, this off-flavour is generally described as acacia blossom, furniture polish, wet wool, mothball, fusel alcohol, and combined with a loss of typical wine bouquet (Hoenicke, Christoph, Schwab, Simat, & Steinhart, 2000; Hoenicke, Borchert, Grüning, & Simat, 2002a).

The detailed formation mechanism of 2-AAP has not been ascertained to date. The concentration of 2-AAP is typically below the odour threshold in the berry, in the must, and in the wine immediately after fermentation. 2-AAP seems to be mainly formed during the wine storage after sulphurization (Christoph et al., 1998; Hoenicke et al., 2002a, 2002b). It has been suggested that the formation of 2-AAP is directly related to the degradation of indole-3-acetic acid (IAA) (Christoph et al., 1998; Hoenicke et al., 2002a).

Research has shown that superoxide radicals are involved in the conversion of IAA to 2-AAP. It is proposed that the oxidation of sulfite induces the formation of radicals and a co-oxidation of IAA which leads to the cleavage

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of the indole ring and subsequent formation of 2-AAP (Hoenicke et al., 2002a). This hypothesis is further supported by the fact that UTA off-flavour has not been reported in red wines, and that red wines spiked with IAA before fermentation have not shown any significant formation of 2-AAP (Christoph et al., 1998). The degradation of IAA to 2-AAP is probably blocked in red wines due to the presence of phenolic radical scavengers (Christoph et al., 1998). Although the anti-oxidative capacity does not correlate with the UTA-intensity of the wine (Hoenicke et al., 2002b), the analysis of grapes musts and their corresponding wines reveals that wines with a higher superoxide radical scavenger activity are less prone to UTA formation (Hoenicke et al., 2002b).

Free IAA and other tryptophan metabolites are typically present at minute amounts in grape must, and more than 95% of the total IAA are bound either as ester conjugate or amide conjugate (Hoenicke, Simat, Steinhart, Köhler, & Arnold, 2001b), or bound by phenolic substances (Schwab, Christoph, Kohler, Gessner, & Simat, 1999). These bound IAA can be converted to the free IAA rapidly during fermentation, and lead to the formation of 2-AAP.

UTA off-flavour seems particularly related to vine stress caused by low rainfall or intensive solar radiation and nutrient deficiencies (Hoenicke et al., 1999). Sensory and chemical analysis has demonstrated that the grape maturity has a significant influence on UTA-intensity and AAP-concentration in the wine (Hoenicke et al., 1999). Wines from early harvest grapes developed significant higher UTA intensities than corresponding wines from late harvest. The combination of early harvest and high grape yield showed a maximum UTA formation (Hoenicke et al., 1999).

The analysis of 2-AAP is complex and difficult due to its low concentration in wines. Dollmann and co-workers (1996) developed a stable isotope dilution analysis technique to quantify 2-AAP in wine after liquid–liquid extraction, Rapp and co-workers (1995) used two dimensional gas chromatography in combination with nitrogen-specific detection to achieve a detection limit of 20 ng/L. The objective of this study was to develop a rapid analytical method to quantify 2-AAP in wine using direct immersion solid-phase microextraction (DI-SPME) and gas chromatography (GC)–mass spectrometry (MS) technique and evaluate the sensory properties of 2-AAP in Chardonnay and Pinot gris wines via flash profile technique.

2. Materials and methods

2.1. Chemicals

2-AAP (⩾98%) and acetophenone-d_8 (AP-d8, internal standard, 98 atom % D) were purchased from Sigma–Aldrich Co. (St. Louis, MO). Ethanol, absolute 200 proof, was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY).

2.2. White wines

Three varieties of white wines, including Chardonnay, Pinot blanc and Pinot gris, were purchased from local grocery stores.

2.3. DI-SPME condition

A 50/30 μm divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS) fibre (2 cm, Supelco Inc., Bellefonte, PA) was used for the extraction of 2-AAP. An aliquot of 15 mL of each wine sample was placed into a 20 mL vial with Teflon-faced silicone septa. The sample was equilibrated at 30 °C for 5 min, and then the SPME fibre was directly immerged into the solution for 30 min at the same temperature under stirring. After extraction, the fibre was inserted into the injection port of the GC (250 °C) to desorb the analytes.

2.4. Gas chromatography–mass spectrometry

Capillary GC–MS was carried out using an Agilent 6890 GC with a 5973 mass selective detector (Agilent Technology, Palo Alto, CA). The sample was analyzed on a HP-5 column (30 m × 0.32 mm i.d., 0.25 μm film thicknesses, Agilent Technology). The carrier gas was helium at a constant flow rate of 2 mL/min. The oven temperature was initially at 40 °C, and then increased to a final temperature of 250 °C at a rate of 10 °C/min. The injector temperature was 250 °C. The splitless mode was used. The SPME fibre was remained in the injector until the completion of analysis. The electron impact energy of the MSD was 70 eV, and the ion source temperature was set at 230 °C. The mass spectra were obtained from a scan range of 50–140.

2.5. Quantification of 2-AAP

A synthetic wine was prepared according to the literature with some modifications (Mestres, Busto, & Guasch, 1998). L-Tartaric acid (3.5 g) was dissolved into 1 L of 12% (by volume) ethanol solution, and pH was adjusted to 3.5 with 1 M NaOH.

A set of synthetic wines (15 mL) containing 5 different concentrations of 2-AAP (from 0.001, 0.1, 0.5, 1, and 4 μg/L) and 1 μg/L AP-d8 were placed into 20 mL vial with Teflon-faced silicon septa. 2-AAP and the internal standard were extracted with the SPME fibre and analyzed using GC–MS as described previously. The m/z of 110 for 2-AAP and m/z of 120 for internal standard (AP-d8) were used for quantification. The standard curve for 2-AAP was built up by plotting the response ratio of 2-AAP to AP-d8 against the concentration ratio using Chemstation software.

Wine sample (15 mL) was placed into 20 mL vial with Teflon-faced silicone septa, and then spiked with AP-d8 solution to give a final concentration of 1 μg/L. The spiked

wine sample was analyzed with the described DI-SPME and GC–MS method.

Known amounts of 2-AAP were added to synthetic wine, as well as in Chardonnay, Pinot blanc, and Pinot gris wines, respectively. The concentrations of 2-AAP in these wines before and after addition of 2-AAP were quantified by the procedure described previously. The recovery rates of 2-AAP in these wines were calculated.

$$\text{Recovery} = (\text{detected amount after addition} - \text{detected amount before addition}) \times 100\%/\text{added amount}$$

2.6. Sensory evaluation

Eight panelists (5 males and 3 females), whose ages were from 22 to 45, were graduate students and researchers in the Department of Food Science and Technology at Oregon State University. All of them had prior sensory training and experiences in wine evaluation.

Two white wines, Pinot gris and Chardonnay, were analyzed first to determine 2-AAP concentration in the original wines, then the wine samples were spiked with 2-AAP to give final concentrations of 0.02, 0.05, 0.1, 0.5 and 1 μg/L. Sensory characteristics of spiked Chardonnay and Pinot gris wines were investigated separately using a flash profile method. Five 30-min sessions were used for each wine variety. During the first session, all panelists were given a brief outline of the procedures (Dairou & Sieffermann, 2002; Delarue & Sieffermann, 2004). Panelists were introduced to the samples spiked with 2-AAP. After smelling all 6 samples, they were asked to create their own lists of descriptors which can best differentiate the wine samples. In the next session, they smelled the same 6 samples again and refined their lists. The following three sessions were test sessions and each test session was a replicate. During each session, panelists received all six samples at the same time. They were instructed to smell the samples in random order and then rank samples based on the intensity of their own descriptors. They were allowed to evaluate samples in their own pace and take enough time to rest. Finally, they recorded the descriptors' ranking scores (1 = weakest and 6 = strongest intensity) of each sample on ballot.

One-way analysis of variance (ANOVA) was first performed on each descriptor within a panelist to examine if the descriptor can be used to significantly discriminate six wine samples with various levels of 2-AAP by the particular panelist (Dairou & Sieffermann, 2002). Descriptors which cannot significantly discriminate wine samples ($p > 0.1$) were excluded in the further analysis. Panelist repeatability was examined. The data from six panelists with their significant descriptors were first transformed by generalized procrustes analysis (GPA). Then discriminant analysis was performed on transformed data within each panelist and concentration was used as a grouping factor to evaluate whether the three wine replicates can be classified together.

After examining the repeatability of the panelists, the untransformed data were used to obtain the mean value across three replications. GPA on these mean data was used to provide a consensus configuration to understand the relationship of sensory properties of Chardonnay and Pinot gris with various concentrations of 2-AAP. Cluster analysis (k-mean) was performed after GPA on descriptors to help semantic explanation (Dairou & Sieffermann, 2002). Discriminant analysis, cluster analysis and one-way analysis of variance were performed using SPSS 11.0 for windows (SPSS Inc., Chicago, Illinois). GPA was performed using Senstools 2.3 (OP&P Product Research BV, Utrecht, The Netherlands).

3. Results and discussion

3.1. Determination of 2-AAP in wine

To develop a suitable SMPE method to analyze 2-AAP in wine, many parameters that influence SPME extraction efficiency need to be carefully selected. Headspace-SPME technique and direct immersion SPME technique (DI-SPME) were compared first. The experiment was conducted using DVB/CAR/PDMS fibre in synthetic wine (spiked with 2 ppb of 2-AAP). Under the same experimental conditions (15 mL sample in a 20 mL vial, saturated with NaCl, pre-equilibrated at 50 °C for 5 min, and then extracted at the same temperature for 30 min), the peak area of 2-AAP by DI-SPME extraction was six times that of HS-SPME extraction. The extraction temperature 30 °C, 40 °C and 50 °C were studied for DI-SPME technique. The results showed that the response of 2-AAP was the highest when extracted at 30 °C. The spiked wine sample was extracted for 20 min, 30 min and 40 min. It was observed that extraction efficiency for 2-AAP increased with extraction time, but the change was relatively small from 30 min to 40 min. Thus, the extraction time was selected for 30 min to shorten the sample preparation time. Although the optimization of SPME extraction parameters were not systematically performed, the parameters obtained from the screening experiment seemed to be suitable for 2-AAP analysis.

Stable isotope dilution technique is most frequently used to quantify aroma compounds at extremely low concentration. However, the synthesis and purification of the stable isotope compound is tedious and expensive. In this study, baseline separation of 2-AAP was achieved chromatographically, the standard curve was built in the synthetic wine, so the internal standard can be easily chosen as long as the wine samples do not contain the internal standard and the quantifying ion was not interfered by other components. 4-Aminoacetophenone was tested first but the elution time on the GC column is too long. Acetophenone was well separated chromatographically, however, some samples contained low concentrations of this compound.

Table 1
Concentration of 2-AAP in commercial white wine samples ($n = 3$)

Cultivar[a]	Vintage[b]	Region	Concentration (ng/L)
Pinot gris-A	2003	California, US	4.6 ± 1.8
Pinot gris-B	2002	Canada	11.3 ± 3.4
Pinot blanc-C	2003	Oregon, US	4.0 ± 2.1
Pinot blanc-D	2003	Oregon, US	3.4 ± 1.1
Pinot blanc-C	2002	Oregon, US	5.4 ± 3.6
Pinot blanc-D	2002	Oregon, US	7.3 ± 2.4
Pinot blanc-C	1999a	Oregon, US	13.7 ± 4.6
Pinot blanc-C	1999b	Oregon, US	12.3 ± 2.6
Chardonnay-F	2003	California, US	4.9 ± 2.9
Chardonnay-G	2003	Oregon, US	<1
Chardonnay-E	2002	California, US	1.9 ± 0.6
Pinot gris-F	2003	Oregon, US	<1
Pinot gris-D	2002	Oregon, US	3.1 ± 0.5

[a] A, B, C, D, E, F, and G were the brand of white wine.
[b] a, b indicated the different batch of white wine at the same brand.

Since the stable-isotope acetophone-d8 (AP-d8) is readily available commercially, it was used as the internal standard.

Different concentration of 2-AAP and internal standard AP-d8 were spiked into synthetic wines to build calibration curve. The linear response was obtained from 0.001 μg/L to 4 μg/L with a correlation coefficient (R^2) greater than 0.99. The recovery rates of 2-AAP in different wine matrices were studied. Known amounts of 2-AAP was added to 4 different wines and the synthetic wine. The concentrations were measured before and after the spiking of 2-AAP. The recovery rate was 97% in synthetic wine, 80–90% in Chardonnay and Pinot gris, and 70–80% in Pinot blanc. The lower recovery in some wines could be due to the interaction of 2-AAP with the wine matrix.

Table 2
Percentage of good reattribution of panelist on evaluation of Chardonnay and Pinot gris spiked with 2-AAP

Wine	Panelist					
	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
Chardonnay	77.8	77.8	88.9	77.8	83.3	72.2
Pinot gris	94.4	72.2	100	88.9	88.9	72.2

Thirteen commercial wines were obtained from local stores, and an informal sensory screening test did not identify any UTA off-flavour. The concentrations of 2-AAP were analyzed in all samples, and the results were listed in Table 1. As shown in the table, the concentration of 2-AAP in those wines varied widely, the highest concentration was 14 ng/L.

3.2. Sensory evaluation

A flash profile technique was used to study the sensory impact of 2-AAP on the aroma of Chardonnay and Pinot

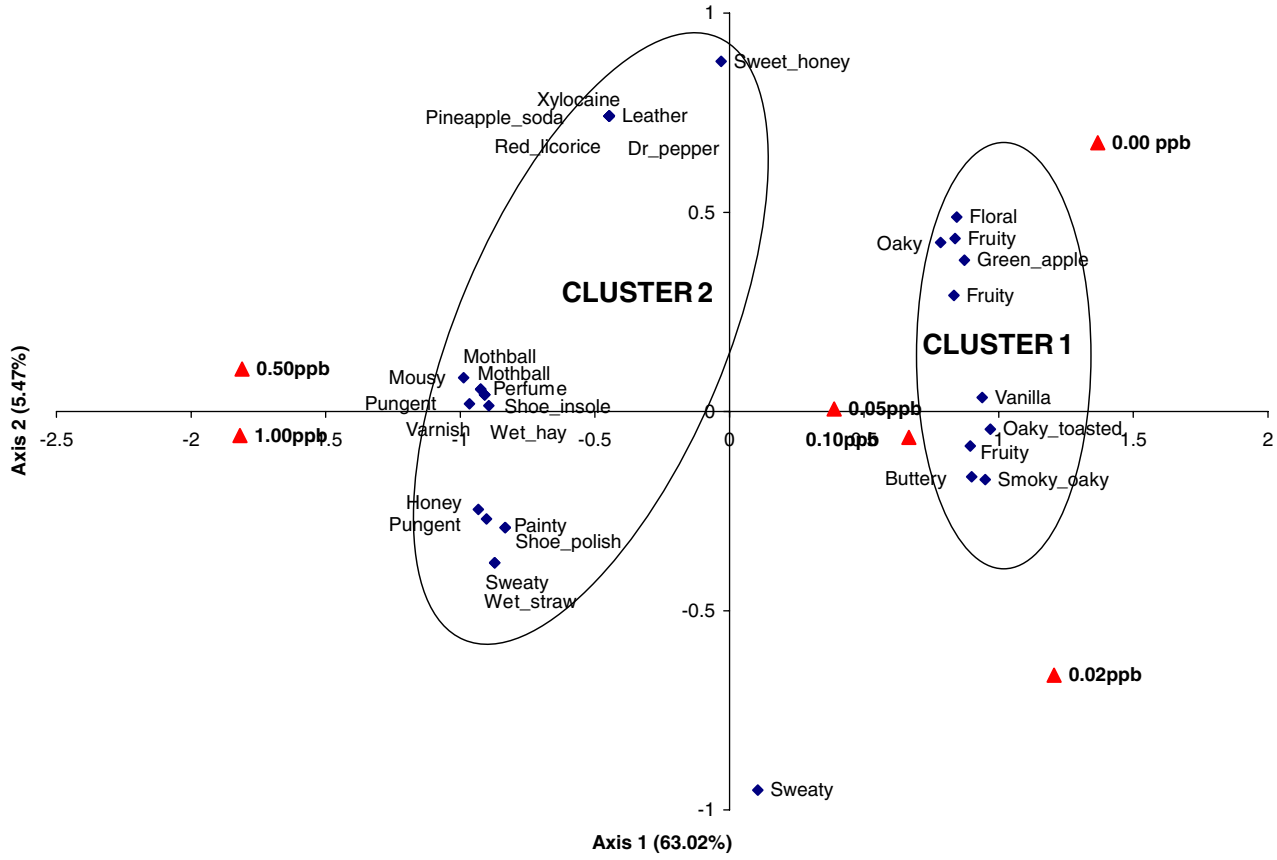

Fig. 1. GPA results on spiked Chardonnay data. There were 6 samples spiked with 0, 0.02, 0.05, 0.1, 0.5, and 1.0 μg/L. The free-choice descriptors were grouped into 2 clusters (indicated by 2 circles).

gris. This method is relatively new and rapid, and it could replace traditional, time-consuming descriptive analysis to provide a snap view of the sensory properties. Its major advantage is that panelists do not need long-time training. Flash profile is based on the principle of free-choice profiling but uses a ranking (instead of assigning intensity scores) to evaluate all samples simultaneously (Dairou & Sieffermann, 2002, 2004).

Eight panelists participated in the sensory test. In the first session, each subject created more than 6 descriptors to differentiate wine samples at different 2-AAP levels. During refining process, subjects were instructed to choose between 3 and 9 descriptors to form their final lists and used them in sample evaluation. The one-way ANOVA (for parametric data) was performed on each descriptor within a panelist to examine inconsistencies among the attributes used by subjects because the Friedman's test cannot handle replications. According to Dairou and Sieffermann (2002), the results may provide valid measurements when the one-way ANOVA was used to test discrimination ability of the attributes. After the analyses, descriptors generated from two panelists could not significantly discriminate the wine samples ($p > 0.1$) and thus their data were dropped. These two panelists were able to generate their own descriptors to discriminate the wine samples in the training but failed to use these descriptors in the test sessions. This failure could be resulted from olfactory fatigue because the flash profile technique requires panelists to smell and rank all six wine samples simultaneously. The repeatability of the rest of the panelists was good as showed by the percentage of reattribution (Table 2). The higher the percentage was determined, the more reliable the panelist was. Overall over 70% of good reattribution was determined from the remaining panelists, which was generally considered acceptable. Panelists were more reliable in evaluating Pinot gris than Chardonnay, possibly because the Chardonnay wine used in this study had more complex aroma than the Pinot gris, and the complex background aroma interfered the evaluation of Chardonnay.

In total, there were 36 terms created for Chardonnay and 40 terms created for Pinot gris. After performing one-way analysis of variance on the sensory ranking data, 5 terms for Chardonnay and 8 terms for Pinot gris failed to differentiate sample difference ($p > 0.1$) and thus were dropped. The free-choice terms that best differentiated the six 2-AAP spiked Chardonnay and Pinot gris samples

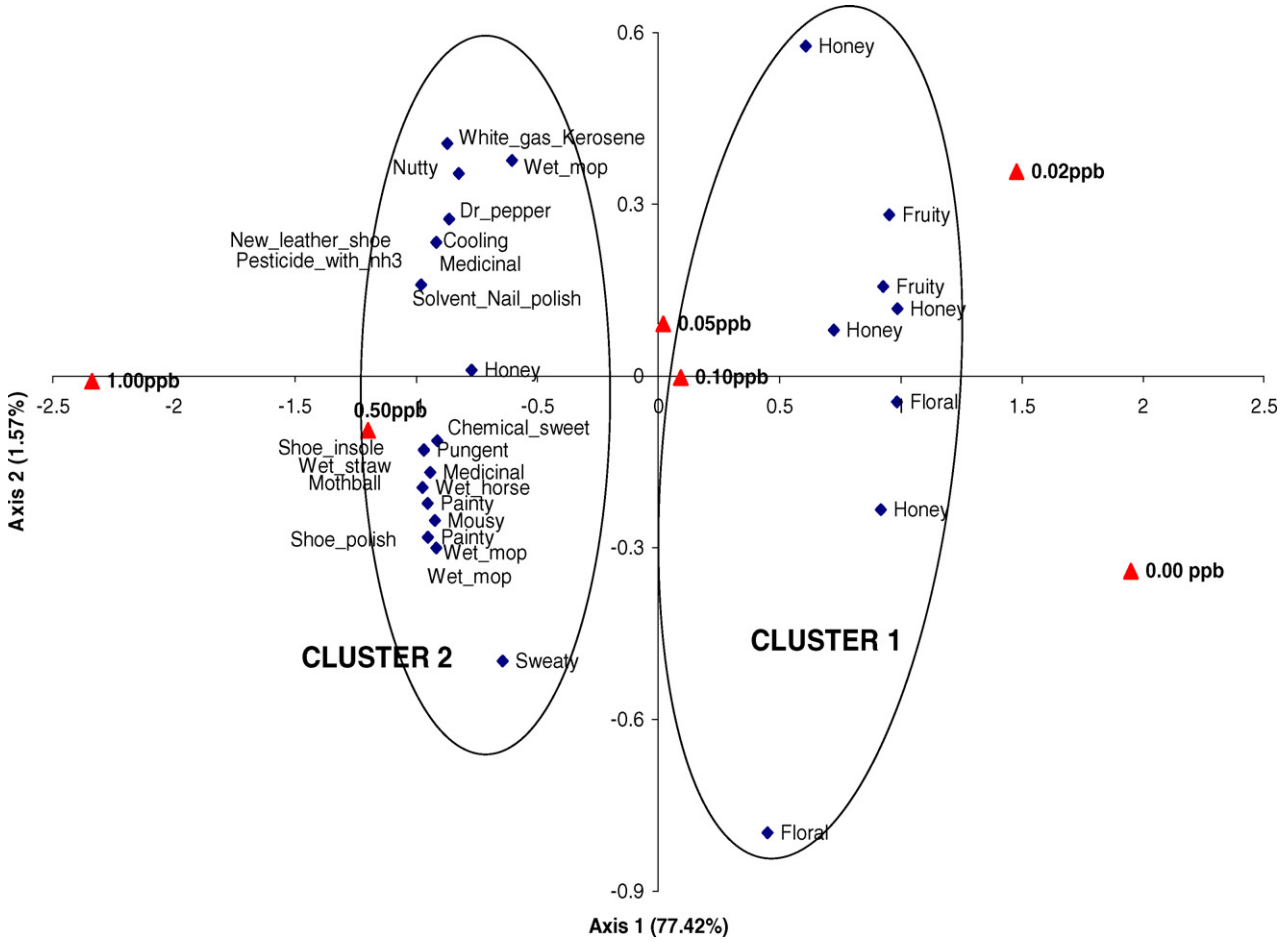

Fig. 2. GPA results on spiked Pinot gris data. There were 6 samples spiked with 0, 0.02, 0.05, 0.1, 0.5, and 1.0 μg/L. The free-choice descriptors were grouped into 2 clusters (indicated by 2 circles).

by the panelists (one-way analysis of variance, $p < 0.1$) were shown in Figs. 1 and 2.

Cluster analyses further separated the descriptors into two categories. Cluster 1 represented wine varietal aromas, including fruity, floral for both wines, green apple, oaky, buttery, smoky/oaky, vanilla, oaky/toasted for Chardonnay, and honey for Pinot gris only. Cluster 2 contained painty, sweaty, Dr. Pepper, honey, mothball, mousy, shoe polish, shoe insole, wet straw for both wines, leather, perfume, pineapple soda, red licorice, sweet/honey, varnish and wet hay for Chardonnay only, while cooling, chemical sweet, gas/kerosene, medicine, solvent/nail polish, new leather shoe, nutty, pesticide, wet mop and wet horse for Pinot gris only. Most descriptors in this cluster were associated with wine off-aromas.

GPA results revealed that aromas of spiked Chardonnay and Pinot gris were shifted gradually from the cluster 1 to the cluster 2 descriptors as the concentration of 2-AAP increased from 0.02 to 1.0 μg/L (Figs. 1 and 2). There were 68.49% (Chardonnay) and 78.99% (Pinot gris) of total variances explained by the GPA after the first two dimensions were extracted, and the Axis 1 shared most explained variances (63.02% in Chardonnay and 77.42% in Pinot gris). The Clusters 1 and 2 descriptors were clearly separated by the Axis 1 and so as the four samples with the lowest spiked 2-AAP and the two with the highest. Chardonnay samples containing 0.02, 0.05, and 0.1 μg/L of 2-AAP had higher intensity ranking in wine-associated aromas (cluster 1) but lower in cluster 2 aromas than those containing 0.5 and 1.0 μg/L of 2-AAP. A similar trend was observed in Pinot gris samples; moreover, more sample separation occurred among samples with two lowest concentrations (<0.01 and 0.02 μg/L), two medium concentrations (0.05 and 0.1 μg/L) and two highest concentrations (0.5 and 1.0 μg/L). Pinot gris with 1.0 μg/L of 2-AAP had strongest cluster 2 aromas and the weakest cluster 1 aromas, followed by 0.5 μg/L (second highest intensity in cluster 2 and the second lowest in cluster 1 aromas). Those with 0.05 and 0.1 μg/L of 2-AAP had all aroma descriptors in moderate levels. While Pinot gris samples containing <0.01 and 0.02 μg/L of 2-AAP had the strongest cluster 1 and the weakest cluster 2 aroma characteristics. Both results demonstrated that cluster 2 descriptors were strongly associated with the occurrence of 2-AAP. The observation that more sample separation occurred in Pinot gris than Chardonnay could result from the different aroma compositions between two wines. Chardonnay samples used in this study had more complex aroma characteristics than Pinot gris, therefore, more complex background aroma in this wine could mask 2-AAP off-flavour. Overall, the UTA off-flavour was detected when the concentration of 2-AAP in the wine reached 0.5 μg/L, this observation was consistent with the sensory studies conducted for Riesling wine which reported a sensory threshold of 0.7–2.0 μg/L for 2-AAP (Hoenicke, Simat, Steinhart, Köhler, & Schwab, 2001a; Hoenicke et al., 2002a; Rapp, 1998; Sponholz & Hühn, 1996).

4. Conclusions

Direct immersion-SPME GC–MS method can rapidly and accurately determine the concentration of 2-AAP in white wines with low detection limit. The method is efficient without tedious sample extraction and the analysis can be completed in only 40 min. The recovery is adequate for trace analysis, and high sensitivity (1 ppt) is achieved without any sample dilution and manipulation. Flash profiling successfully generated aroma descriptors which can differentiate Chardonnay and Pinot gris wines with various levels of 2-AAP. Positive wine aroma gradually disappears concomitantly with the occurrence of negative off-flavour such as mothball, paint, and shoe insole as the spiked 2-AAP concentration increases. The distinct off-flavour can be detected when the concentration of 2-AAP reaches 0.5 and 1.0 μg/L in Chardonnay and Pinot gris, respectively.

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References

Buttery, R. G., & Ling, L. C. (1994). Importance of 2-aminoacetophenone to the flavor of masa corn flour products. *Journal of Agricultural and Food Chemistry, 42*, 1–2.

Christoph, N., Bauer-Christoph, C., Gessner, M., Koehler, H. J., Simat, T. J., & Hoenicke, K. (1998). Formation of 2-aminoacetophenone and formylaminoacetophenone in wine by reaction of sulfurous acid with indole-3-acetic acid. *Wein-Wissenschaft, 53*, 79–86.

Culleré, L., Escudero, A., Cacho, J. F., & Ferreira, V. (2004). Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry, 52*, 1653–1660.

Dairou, V., & Sieffermann, J.-M. (2002). A comparison of 14 jams characterized by conventional profile and a quick original method, the flash profile. *Journal of Food Science, 67*, 826–834.

Delarue, J., & Sieffermann, J.-M. (2004). Sensory mapping using flash profile. Comparison with a conventional descriptive method for the evaluation of the flavor of fruit dairy products. *Food Quality and Preference, 15*, 383–392.

Dollmann, B., Wichmann, D., Schmitt, A., Koehler, H., & Schreier, P. (1996). Quantitative analysis of 2-aminoacetophenone in off-flavored wines by stable isotope dilution assay. *Journal of AOAC International, 79*, 583–586.

Ferreira, V., Ortín, N., Escudero, A., López, R., & Cacho, J. (2002). Chemical characterization of the aroma of Grenache Rosé wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *Journal of Agricultural and Food Chemistry, 50*, 4048–4054.

Hoenicke, K., Simat, T. J., Steinhart, H., Christoph, N., Kohler, H. J., & Schwab, A. (1999). Determination of tryptophan and tryptophan metabolites in grape must and wine. *Advances in Experimental Medical Biology, 467*, 671–677.

Hoenicke, K., Christoph, N., Schwab, A., Simat, T. J., & Steinhart, H. (2000). The untypical aging off-flavor (UTA) in wine-formation and possible preventions. *Czech Journal of Food Science, 18*, 52–54.

Hoenicke, K., Simat, T. J., Steinhart, H., Köhler, H. J., & Schwab, A. (2001a). Determination of free and conjugated indole-3-acetic acid, tryptophan, and tryptophan metabolites in grape must and wine. *Journal of Agricultural and Food Chemistry, 49*, 5494–5501.

Hoenicke, K., Simat, T. J., Steinhart, H., Köhler, H. J., & Arnold, S. (2001b). Determination of free and conjugated indole-3-acetic acid, tryptophan, and tryptophan metabolites in grape must and wine. *Journal of Agricultural and Food Chemistry, 49*, 5494–5501.

Hoenicke, K., Borchert, O., Grüning, K., & Simat, T. (2002a). Untypical aging off-flavor in wine: Synthesis of potential degradation compounds of indole-3-acetic acid and kynurenine and their evaluation as precursors of 2-aminoacetophenone. *Journal of Agricultural and Food Chemistry, 50*, 4303–4309.

Hoenicke, K., Simat, T. J., Steinhart, H., Christoph, N., Geßner, M., & Köhler, H.-J. (2002b). 'Untypical aging off-flavor' in wine: Formation of 2-aminoacetophenone and evaluation of its influencing factors. *Analytical Chimica Acta, 458*, 29–37.

Karagül-Yüceer, Y., Drake, M., & Cadwallader, K. R. (2001). Aroma-active components of nonfat dry milk. *Journal of Agricultural and Food Chemistry, 49*, 2948–2953.

Karagül-Yüceer, Y., Cadwallader, K. R., & Drake, M. (2002). Volatile flavor components of stored nonfat dry milk. *Journal of Agricultural and Food Chemistry, 50*, 305–312.

Karagül-Yüceer, Y., Vlahovich, K. N., Drake, M., & Cadwallader, K. R. (2003). Characteristic aroma compounds of rennet casein. *Journal of Agricultural and Food Chemistry, 51*, 6797–6801.

Mestres, M., Busto, O., & Guasch, J. (1998). Headspace solid-phase microextraction analysis of volatile sulphides and disulphides in wine aroma. *Journal of Chromatography A, 808*, 211–218.

Preininger, M., & Ullrich, F. (2001). Trace compound analysis for off-flavor characterization of micromilled milk powder. In J. V. Leland et al. (Eds.), *Gas chromatography-olfactometry: The state of the art* (pp. 46–61). Washington, DC: American Chemical Society.

Rapp, A., Versini, G., & Ullemeyer, H. (1993). 2-Aminoacetophenone: The causal agent of the untypical aging flavor (naphthalene note or hybrid note) of wine. *Vitis, 32*, 61–62.

Rapp, A., Versini, G., & Engel, L. (1995). Determination of 2-aminoacetophenone in fermented model wine solutions. *Vitis, 34*, 193–194.

Rapp, A. (1998). Volatile flavor of wine: Correlation between instrumental analysis and sensory perception. *Nahrung, 42*, 351–363.

Schwab, A. L., Christoph, N., Kohler, H.-J., Gessner, M., & Simat, T. (1999). Influence of viticultural treatments on the formation of the untypical aging off-flavour in white wines. Part I: Influence of the harvest time. *Wein-Wissenschaft, 54*, 114–120.

Sponholz, W. -R., & Hühn, T. (1996). Aging of wine: 1,1,6-Trimethyl-1,2-dihydronaphthalene (TDN) and 2-aminoacetophenone. In Henick-Kling, T., Wolf, T. E., & Harkness, E. M. (Eds.), *Proceedings for the 4th international symposium on cool climate viticulture and enology* (pp VI-37–VI-56). Rochester, New York, USA.