Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 99 (2006) 183–190
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

A rapid method for quantifying aroma precursors: Application to grape extract, musts and wines made from several varieties

M. Arévalo Villena [a], J. Díez Pérez [b], J.F. Úbeda [a], E. Navascués [b], A.I. Briones [a,*]

[a] Tecnología de Alimentos, Universidad de Castilla La Mancha, Avda, Camilo José Cela s/n, 13071 Ciudad Real, Spain
[b] Agrovín S.A. Poligono Industrial Los Alces s/n, Alcázar de San Juan, Ciudad Real, Spain

Received 31 January 2005; received in revised form 12 July 2005; accepted 30 July 2005

Abstract

This paper reports on a quick and simple method for reliably quantifying aroma precursors in grape extract, musts and wines of different grape varieties. The method, which is intended mainly for use by wineries and analytical laboratories, is based on isolation of terpenyl-β-D-glycosides through selective retention on a C_{18} reversed-phase column, followed by hydrolysis to liberate terpenes, yielding an equimolar proportion of free aglycons and glucose. Sugar was measured using an enzyme analysis kit. Aroma precursors were quantified using this method, in a range of crushed grapes, musts and wines produced in the Castilla La Mancha region. The method was also applied on a larger scale in a wine analytical laboratory, to chart the behavior of terpenyl glycosides during the final stages of ripening of grapes from different viticulture Spanish regions.

The results obtained provided a reliable indication of the aroma potential of the varieties studied. The method is simple, practical and readily applicable in wineries.

Keywords: Terpenil β-D-glycosides; Aroma precursors quantification; Grape extract; Must; Wine

1. Introduction

The aroma potential of a grape must derives from aromatic free volatiles and from non-volatile, odorless precursors, which may be hydrolyzed during the wine-making process. Over recent years, increasing attention has been paid to these glycosidically bound precursors, since – in certain varieties of must – some monoterpenes, norisoprenoids and shikimic acid derivatives are bound to sugars, and glycosidase-catalyzed hydrolysis releases the volatiles associated with the fruity aroma so highly appreciated in white wines.

The major precursors include structures such as β-D-glucopyranoside, α-L-arabinofuranosyl-β-D-glucopyranoside, α-L-ramno-pyranosyl-β-D-glucopyranoside and β-apiosyl-β-D-glucopyranoside (Gunata, Bitteur, Brillouet, Bayonove, & Cordonnier, 1988; Voirin, Baumes, Bitteur, Gunata, & Bayonove, 1990; Williams, Strauss, Wilson, & Massy-Westropp, 1982).

Quantification of these molecules is of particular interest to winemakers, since they are seen as a quality parameter in white grape varieties used for young fruity wines. It is also of interest when using treatments involving commercial enzyme preparations, in order to establish the right dose of each product to be added, and to predict the outcome of treatment.

Reports indicate that aroma precursor glycosides are not present in all grape varieties, and where present are not always to be found at the same concentrations (Arrhenius, McCloskey, & Sylvan, 1996; Di Stefano, Borsa, Maggiorotto, & Corino, 1995; Garcia-Moruno, Ribaldone, & Stefano, 2000; Gunata, Bayonove, Baumes, & Cordonnier, 1985; Lao, López Tamanes, Lamuela Raventos, Buxaderas, & Torre Boronat,

* Corresponding author. Tel.: +34 926 295300x3424; fax: +34 926 295318.
E-mail address: Ana.Briones@uclm.es (A.I. Briones).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.07.039

1997; McCloskey, Sylvan, & Arrhenius, 1996; Nicolini, Versini, & Adalla, 1993; Razungles, Gunata, Pinatel, Baumes, & Rayonove, 1993; Reyero et al., 2000; Reynolds & Wardle, 1997; Zoecklein, Marcy, Williams, & Jasinski, 1997). Two-thirds of glycosides are found in the must, and the other third in the grape skin; thus they can be almost completely recovered by simple pressing. By contrast, free terpenes and anthocyanins are almost exclusively found in skin, and maceration is thus required for their extraction (Boulton, Singleton, Bisson, & Kunkee, 1998).

Methods used for glycoside analysis generally involve complex procedures combining extraction and detection by gas chromatography (Voirin, Baumes, Gunata, Bitteur, & Bayanove, 1992; Voirin, Baumes, Sapis, & Bayanove, 1992), although Williams et al. (1995), have proposed a very simple method based on quantification of the glucose released through hydrolysis of precursors.

This paper reports on a modification of the method developed by Williams et al. (1995). Since hydrolysis of glycosides yields an equimolar proportion of aglycons and D-glucose, determination of the glucose liberated by hydrolysis will permit an estimation of the total concentration of glycosylated secondary metabolites present in the substrate. This modified method was applied to grape extract, musts and wines (mainly white). This method will provide wineries with a simple, readily applied test to determine the aroma potential of musts and wines and to know the effectiveness of the enzyme treatments used.

2. Materials and methods

The method proposed by Williams et al. (1995) was modified at each stage (glycoside retention on a C_{18} reversed-phase column, washing to remove polar substances, recovery of precursors by elution, acid hydrolysis and measurement of the glucose released). Standard solutions of the synthetic glycoside *N*-octyl β-D-glucoside (Sigma) were used added or not with other compounds depending on the parameter studied.

The measurement method developed as described below was then applied to real samples of grapes, musts and wines, first in our own laboratory and later in a commercial wine analytical laboratory.

In order to identify the appropriate modifications to the original method, the following parameters were examined.

2.1. Column recovery capacity

This was tested using 20 ml of a range of *N*-octyl β-D-glucoside solutions of known concentration (0.00, 0.10, 0.25, 0.50, 1.00 and 1.25 μmol *N*-octyl ml^{-1}).

2.2. Flow rate

A 0.5 μmol ml^{-1} solution of *N*-octyl β-D-glucoside was passed through the column at three different flow rates: low (2 ml min^{-1}), medium (3 ml min^{-1}) and high (4 ml min^{-1}).

2.3. Number of uses per column

Using an *N*-octyl solution (0.2 μmol ml^{-1}), liberated glucose was measured after using the same column 1–4 times.

2.4. Interference of other compounds

The potential interference of flavonols in the determination of aroma precursors was studied, using both synthetic and natural flavonols.

- *Synthetic flavonols*. Various concentrations of the synthetic flavonol quercetin 3-β-D-glucopyranoside (Fluka) (0, 0.03, 0.06 and 0.12 μmol ml^{-1}) were added to a 0.3 μmol ml^{-1} *N*-octyl β-D-glucopyranoside solution, and the amount of glucose released was measured for each assay.
- *Natural flavonols*. A Muscat was used, both at natural pH and at pH 10 adjusted with concentrated NaOH (Iland, Cynkar, Francis, Williams, & Coombe, 1996). Both were eluted through the column and subjected to acid hydrolysis. The pH 10 sample was divided into two fractions: one was used to analyze glucose released after acid hydrolysis, and the other was eluted again; the first milliliter of eluate – containing the flavonols which might interfere in measurements – was discarded.

2.5. Resulting protocol

Modification of the various steps yielded the following quantification protocol:

Step 1, sample preparation.

(a) Musts and wines:. They were centrifuged at 4500 rpm for 5 min.
(b) Grape extract: Grape samples were homogenized using a homogenizer with a dispersing head; 10 g of homogenate were added to 10 ml of a 50% ethanol solution and were agitated at 200 rpm for 2 h at room temperature. Homogenate was then centrifuged at 4500 rpm for 5 min; 5 ml of the clean product were dilute with water to 25 ml in order to obtain the appropriate alcohol concentration (always less than 15% v/v).

Step 2, isolation of glycosides. C_{18} cartridges were pretreated with 10 ml methanol (HPLC quality) followed

by 10 ml Milli-Q water (Williams et al., 1995). An appropriate volume of each sample (15 ml for must and grape extract, and 20 ml for wine) was loaded onto the cartridge. These volumes, which differ from those indicated in the original protocol, were considered more suitable for column retention capacity, because the precursor content in musts is greater than in wine due to collateral hydrolysis of terpenyl glycosides during fermentation. The cartridge was then washed with water (3×15 ml for grape extract and wine, 3×20 ml for must). Glycosides were eluted with 1.5 ml 100% HPLC-quality ethanol followed by 3 ml water, and adjusted to a final volume of 5 ml with water. In all cases, the flow rate was approximately 3 ml min^{-1}.

Step 3, acid hydrolysis. No modification was made to the original method: to aliquots (0.5 ml) of the above glycoside eluate was added H_2SO_4 (1.0 ml, 2.25 M) to give solutions for hydrolysis containing 1.5 M H_2SO_4 and 10% v/v ethanol. A control solution was similarly prepared for each eluate, with water (1.0 ml) added in place of the H_2SO_4 solution, for determination of the free (i.e., non-glycosidic) glucose concentration of the eluate. A reagent blank was made with 30% ethanol in place of the glycoside eluate. The samples and the reagent blank were brought to boiling for 1 h, while controls were held at room temperature.

Step 4, analysis of D-*glucose*. The D-glucose released in each of the assays was measured using a glucose oxidase assay kit (Sigma). Aliquots of the solutions after hydrolysis (262 μl) were transferred to plate wells to which 3 M NaOH solution (260 μl) was added. For the control solutions, water substituted to the NaOH solution. It was found that with 144 μl of a 95 μg ml^{-1} glucose solution all test samples lay within the detection range of the enzyme kit. To 200 μl of sample, 400 μl of the kit solution were added, and the mixture was then held at 37 °C for 30 min; the reaction was stopped by addition of 400 μl 6 M H_2SO_4, following supplier's recommendations. Absorbance was then read at 540 nm, a wavelength absorbing the pinkish color formed by glucose oxidation to glyconic acid and the subsequent reaction with *o*-dianisidine. Resulting values were interpolated into a glucose calibration curve.

2.6. Application to real samples

In all cases, musts and grapes were collected from various different wine cellars and vineyards around the Castilla la Mancha region. The following test samples were used.

- *Grape extract*. Grapes of the following varieties were processed as indicated earlier: Airén, Chardonnay, Chenín, Garnacha, Muscat, Rosanne, Sauvignon blanc and Verdejo.
- *Must and wine*. The following varieties were used: Airén, Chardonnay, Gewürztraminer, Macabeo, Muscat, Sauvignon blanc and Riesling. Musts were fermented in the laboratory in 3-l flasks using the commercial *Saccharomyces cerevisiae* strain UCLM 325 (Biospringer). Fermentation was monitored for weight loss and considered to have finished when the sugar concentration fell below 5 g l^{-1}.
- *Must, wine and enzyme-treated wine*. Three varieties were used: Airén, Macabeo and Muscat. A fraction of the wines were made from each variety were treated with the recommended dose of a commercial enzyme preparation designed specifically to enhance aroma release in white varieties; this preparation was kept in contact with the wine for 15 days at room temperature.

2.7. Application in an analytical laboratory (Agrovin SA, Spain)

The aim was to ascertain whether this method was routinely applicable at a larger scale in an analytical laboratory receiving samples from different Spanish regions (Galicia, Andalucía, Castilla la Mancha,and Castilla León). Aroma precursors were measured in the following varieties: Muscat, Albariño, Macabeo (irrigated), Airén (irrigated and rainfed), Gewürztraminer and Chardonnay. Precursor levels were also charted over the last 15 days of ripening prior to harvesting.

All assays were performed in triplicate.

2.8. Statistical analysis

A one-way analysis of variance was performed, using the SPSS statistical software package, to detect significant differences between samples both for the assays carried out when developing the method and those performed when applying the method to real samples.

3. Results and discussion

3.1. Column recovery capacity

The retention capacity of the column was tested using *N*-octyl β-D-glucoside solutions of known concentration, covering the range for the various varieties assayed. Total glucose liberated from known concentrations was calculated, and the result was compared to that obtained applying the precursor recovery and quantification protocol. As Fig. 1 shows, the percentage recovery was excellent; the linear correlation coefficient (r^2) of 0.996, recorded even with very high glycoside concentrations, suggests that the method effectively addressed this problem and was applicable to any grape variety.

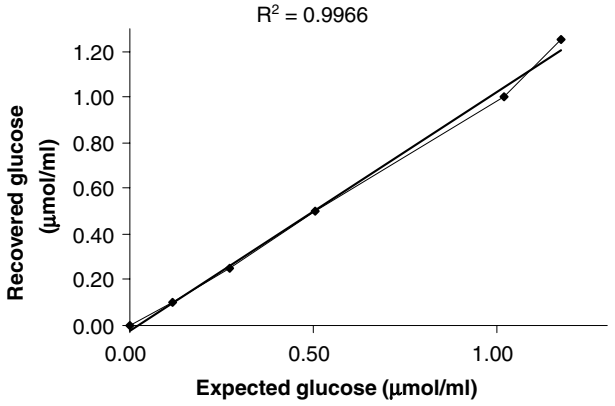

Fig. 1. Recovery of *N*-octyl-β-D-glycoside solutions of known concentration (0.00, 0.10, 0.25, 0.50, 1.00, 1.25 µmol *N*-octyl ml^{-1}).

3.2. Flow rate

A medium concentration of *N*-octyl β-D-glucoside (0.5 µmol ml^{-1}) was used to measure glucose released at the three different flow rates indicated under Section 2 (2–4 ml min^{-1}). Measured glucose ranged from 0.49 to 0.51 µmol min^{-1}. One-way ANOVA indicated no significant differences (95% confidence) between the values obtained at different flow rates and the expected real value. This is of methodological importance, since it allows the analyst a certain margin in this parameter, which is difficult to control.

3.3. Number of uses per column

The *N*-octyl β-D-glucoside concentration used was that most commonly recorded for precursor concentrations (0.2 µmol ml^{-1}). Liberated glucose was measured from four equal samples run on the same column. Table 1 shows mean glucose values obtained for each use of the column. Recovery rates were good until the third use, falling off considerably on the fourth use. ANOVA showed significant differences (95% confidence) between the amount of glucose recovered on the fourth use of the column and the real expected value, while no significant difference was found between the real value and the values recorded for the first, second and third runs on the same column. This suggests that each column may be used up to three times with 95% reliability, which represents an economic advantage for wineries.

Table 1
Glucose released from a 0.2 µM *N*-octyl β-D-glucoside solution after repeated use of the same column

Number of uses	1	2	3	4
Glucose (µmol ml^{-1})	0.20	0.20	0.22	0.10*

* Significant differences at 95% confidence.

3.4. Interference of other compounds

- *Synthetic flavonols*. Rising concentrations of quercetin 3-β-D-glucopyranoside (0, 0.03, 0.06 and 0.12 µmol ml^{-1}) were added to a 0.3 µmol ml^{-1} *N*-octyl solution, and the amount of glucose released by acid hydrolysis following isolation on C18 columns was measured for each assay. Table 2 shows mean values obtained for glucose released by the various samples. ANOVA showed that quan- tification of the fourth solution (0.12 µM) was significantly lower (95% confidence), suggesting interference at that concentration with the enzyme reaction color development. This however, poses no problem since that flavonol concentration is never reached in real samples (Boulton et al., 1998).
- *Natural flavonols*. Muscat must samples were treated as indicated under Section 2. Table 3 shows mean precursor enzyme values expressed as amount of *N*-octyl ml^{-1} of must. ANOVA showed no significant differences between the various samples assayed, confirming – as in the previous test – that flavonols did not interfere with precursor quantification in real samples.

False positives, i.e., the recovery and quantification of flavonols as though they were aroma precursors, was a potential source of interference due to the structural similarity between the two types of molecules. No false positives were recorded.

3.5. Application to real samples

- *Grape extract*. Grapes of the following varieties were processed as indicated under Section 2: Airén, Chardonnay, Chenín, Garnacha, Muscat, Rosanne,

Table 2
Glucose released from 0.3 µmol ml^{-1} *N*-octyl solutions containing different concentrations of quercetin 3-β-D-glucopyranoside

Sample	A	B	C	D
Glucose (µmol ml^{-1})	0.28	0.29	0.28	0.25*

A, 0.00 µmol ml^{-1}; B, 0.03 µmol ml^{-1}; C, 0.06 µmol ml^{-1}; D, 0.12 µmol ml^{-1}.
* Significant differences at 95% confidence.

Table 3
Concentration of aroma precursors expressed as nmol glucose ml^{-1} must released from terpenyl glycosides in Muscat must treated in different conditions to remove flavonols

Sample	A	B	C
Glucose (nmol ml^{-1})	252.18	260.58	260.58

A, glycosides from must at natural pH; B, glycosides from must at pH 10; C, glycosides from must at pH 10 discarding the first milliliter on a second run.

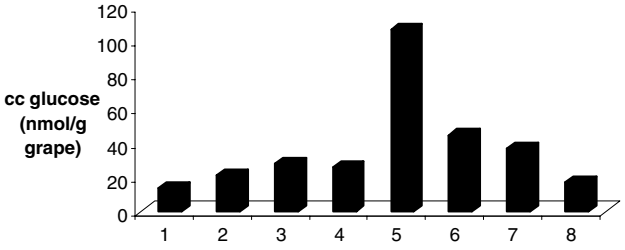

Fig. 2. Aroma precursor concentrations (expressed as nmol glucose g^{-1} grape) in grapes of different varieties: Airén (1); Chardonnay (2); Chenín (3); Garnacha (4); Muscat (5); Rosanne (6); Sauvignon blanc (7); Verdejo (8).

Sauvignon blanc and Verdejo. Fig. 2 shows the glucose measurements obtained following hydrolysis of the glycosides isolated. One-way ANOVA showed significant differences (95% confidence) between all varieties, with the exception of Chenín and Garnacha, which – though similar to each other – differed significantly from the other varieties. As the figure shows, Muscat was the most aromatic variety, and Airén the least aromatic; this confirms results reported in the literature (Boulton et al., 1998; Gunata, Bayonove, Baumes, & Cordonnier, 1986; Iland et al., 1996; Tingle & Halvorson, 1971; Williams et al., 1995).

- *Must and wine.* The following varieties were used: Airén, Chardonnay, Gewürztraminer, Macabeo, Muscat, Saougvinon blanc and Riesling. Precursors were measured in musts and corresponding wines produced in the laboratory after fermentation with *S. cerevisiae* UCLM 325 strain. Fig. 3 shows, again, the considerable variation in precursor concentrations from one variety to another; the ANOVA showed significant differences (95% confidence) in glycosilated terpene levels between all must varieties. Similar results were recorded for wines, except that no significant difference was found between Airén and Saougvinon blanc. The highest value was found for Gewürztraminer, followed closely by Muscat. Riesling also displayed high aroma precursor concentrations, whereas the lowest values were again found for Airén. The decline in precursor levels following fermentation of some varieties may have been due to the residual glucanase activity of certain *S. cerevisiae* strain used (Arévalo Villena, 2003; Boulton et al., 1998; Cordero Otero et al., 2003; Hernández, Espinosa, Fernández-González, & Briones, 2002).It is perhaps surprising that so many studies address the use of enzyme treatments to enhance aroma release (Charoenchai, Fleet, Henschke, & Todd, 1997; Delcroix, Günata, Sapis, Salmon, & Bayonove, 1994; Fernández, Úbeda, & Briones, 2000; Gunata, Bayonove, Tapiero, & Cordonnier, 1990; Manzanares, Rojas, Genovés, & Vallés, 2000; Mendes-Ferreira, Clímaco, & Mendes Faia, 2001; Strauss, Jolly, Lambrechts, & van Rensburg, 2001), given the large amount released during fermentation, particularly considering the low detection threshold of this type of compound, which should permit their complete detection in the final product. However, it would appear that despite the release observed through precursor measurement, there is little organoleptic difference; this may be because during fermentation most of these terpenes are volatilized, drawn off by the CO_2 produced during the process, and lost during the metabolite exchange taking place between the fermentation medium and the atmosphere. In any case, the loss is not a source of concern, since – as the figure shows – in some varieties a large amount of substrate remains, and can be released by enzyme treatment.
- *Must, wine and enzyme-treated wine.* In view of the results obtained in the previous section a new set of musts were analyzed, as were the wines produced from them, some of which were then subjected to a commercial enzyme treatment designed specifically to enhance aroma release. Three varieties were used for this purpose: Airén, Macabeo and Muscat. As Fig. 4 shows, the highest values were again displayed by Muscat, followed by Macabeo and Airén. One-way ANOVA was applied separately to each

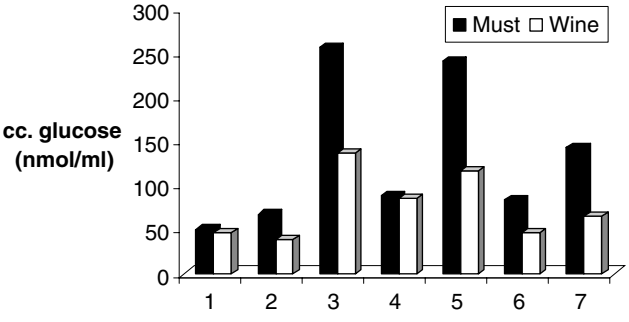

Fig. 3. Aroma precursor concentrations (expressed as nmol glucose ml^{-1}) in musts and wines of different varieties: Airén (1); Chardonnay (2); Gewürztraminer (3); Macabeo (4); Muscat (5); Sauvignon blanc (6); Riesling (7).

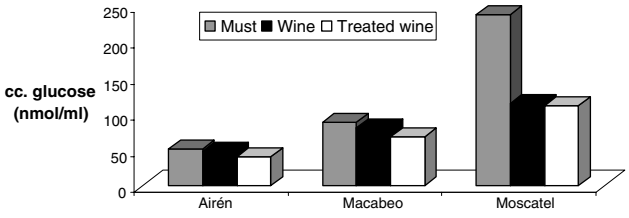

Fig. 4. Aroma precursor concentrations (expressed as nmol glucose ml^{-1}) in musts, untreated wines and enzyme-treated wines of different varieties.

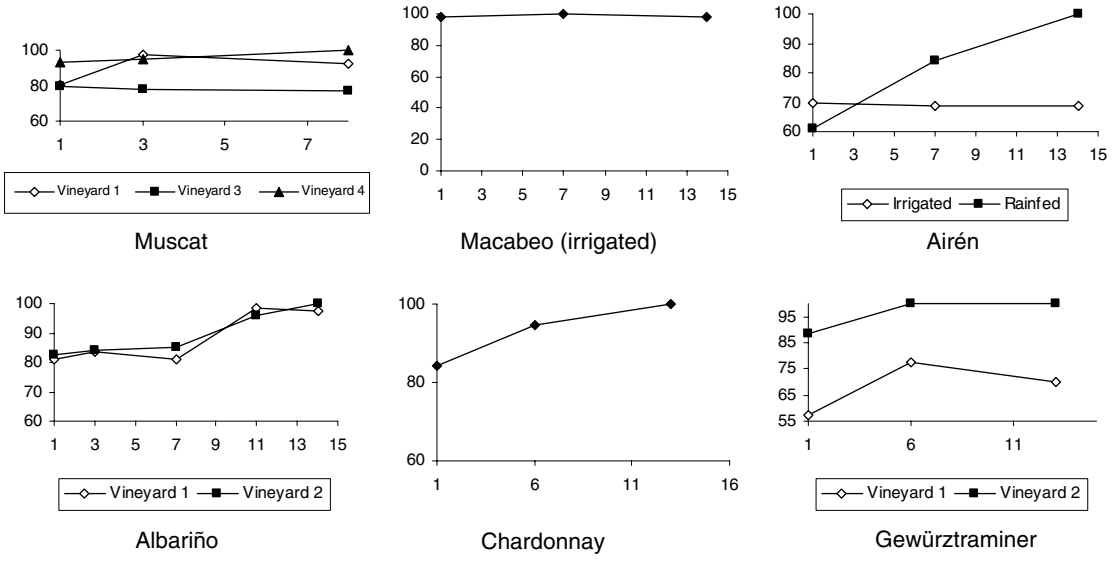

Fig. 5. Behavior of aroma precursors during the days prior to harvesting, expressed as % glucose released as a function of time (days).

variety to detect significant differences (95% confidence) between must, untreated wine and treated wine. The three products differed significantly for Airén, although since this is a neutral variety, this does not necessarily ensure a difference in sensory perception. For Macabeo products, aroma precursor concentrations were significantly higher in enzyme-treated than in untreated wine, suggesting an appropriate use of enzyme treatment; no difference was found between must and untreated wine, presumably because yeast had no significant effect on terpenyl glycosides for this variety. In Muscat, by contrast, significant differences were detected between must and wines (treated and untreated), but not between treated and untreated wines, suggesting that enzyme treatment is unnecessary with this variety. However, these results need to be confirmed by organoleptic testing, since each terpene has a a different detection threshold, and it is the interaction between terpenes that characterizes each variety (Ribereau-Gayon, Glories, Maujena, & Dubourdieu, 2000).

3.6. Application in an analytical laboratory

In order to ascertain whether this method was routinely applicable on a larger scale, it was tested in a wine analytical laboratory, using the following varieties: Muscat, Macabeo (irrigated), Airén (irrigated and rainfed), Albariño, Chardonnay and Gewürztraminer. Grapes were from vineyards in Pontevedra, Málaga, Cuenca, Huesca and Burgos, Aragon sampled over the 15 days immediately prior to harvesting. Fig. 5 shows the behavior of aroma precursors of each variety. Precursor concentrations were expressed as percentage glucose released as a function of time; the last day in each case corresponded to harvesting.

Fig. 5 shows precursor concentrations in different samples of Muscat, Macabeo and Airén. For Muscat, vineyard 1 displayed a slight increase in concentrations towards the end of ripening, while precursor levels in the other two vineyards remained stable. According to the laboratory, the only difference between vineyards 3 and 4, is that vines in vineyard 4 were trained up tall frames; this method of training may thus promote aroma precursor formation when other conditions are constant. Macabeo precursor concentrations remained constant throughout the period studied. The only difference between the two Airén samples was that one was from an irrigated, and the other from a rainfed, vineyard. In the irrigated vineyard, precursors had already formed by the start of the study, whereas samples from the rainfed vineyard displayed a marked increase in precursor levels during ripening, and higher final concentrations. The same figure shows the results for Albariño, Chardonnay and Gewürztraminer, for which the laboratory provided no vineyard information. All three varieties displayed a slight increase in precursor concentrations in the final stages of ripening. A difference was noted in precursor levels between the two Gewürztraminer vineyards.

4. Conclusion

The results obtained here provide a reliable indication of the aroma potential of the varieties studied. The method is simple, practical and readily applicable in wineries, allowing wine-makers to determine aroma

precursor levels in grapes, musts and/or wines, to chart their behavior over the days immediately prior to harvesting and to test the effectiveness of any enzyme treatments used. The method will thus be valuable when taking decisions on the timing of harvesting and/or the use of enzyme treatments (dose, duration) applied to enhance aroma release.

Acknowledgments

The authors are grateful to the Spanish Ministry of Education and Science for an FPU Grant to María Arévalo Villena and for project funding (AGL 2004-05242-C03-01).

References

Arévalo Villena María. (2003). Propuesta de un método rápido para la cuantificación de la actividad β-glucosidásica en levaduras vínicas. *Diploma de Estudios Avanzados*. UCLM.

Arrhenius, S. P., McCloskey, L. P., & Sylvan, M. (1996). Chemical markers for aroma of *Vitis vinifera* var. Chardonnay regional wines. *Journal of Agricultural and Food Chemistry, 44*, 1085–1090.

Boulton, R., Singleton, V., Bisson, L., & Kunkee, R. (1998). Principles and practices of winemaking. Germantown: Aspen Publishers Inc..

Charoenchai, C., Fleet, G. H., Henschke, P. A., & Todd, B. E. N. (1997). Screening of non-*Saccharomyces* wine yeasts for the presence of extracelullar hydrolytic enzymes. *Australian Journal of Grape and Wine Research, 3*, 2–8.

Cordero Otero, R., Úbeda Iranzo, J., Briones Pérez, A., Potgieter, N., Arévalo Villena, M., Pretorious, S., et al. (2003). Characterization of β-glucosidase activity produced by enological strains of non-*Saccharomyces* yeasts. *Journal of Food Science, 68*, 2564–2569.

Delcroix, A., Günata, Z., Sapis, J. C., Salmon, J. M., & Bayonove, C. (1994). Glycosidase activities of three enological yeast strains during winemaking, effect on the terpenol content of Muscat wine. *American Journal of Enology and Viticulture, 45*, 291–296.

Di Stefano, R., Borsa, D., Maggiorotto, G., & Corino, L. (1995). Terpenes and polyphenols in colored aromatic grapes grown in Piedmont. *Enotecnico, 31*, 75–85.

Fernández, M., Úbeda, J. F., & Briones, A. I. (2000). Typing of non-*Saccharomyces* yeasts with enzymatic activities of interest in winemaking. *International Journal of Food Microbiology, 59*, 29–36.

Garcia-Moruno, E., Ribaldone, M., & Stefano, R.-di. (2000). Hydrolysis of terpene glycosides of grape skins in the preparation of base musts from aromatic red-berried grapes. *Rivista di Viticoltura e di Enologia, 53*(1), 27–36.

Gunata, Y. Z., Bayonove, C. L., Baumes, R. L., & Cordonnier, R. E. (1985). The aroma of grapes. Extraction and determination of free and glycosidically bound fractions of some grapes aroma components. *Journal of Chromatography Science, 331*, 83–90.

Gunata, Z., Bayonove, C., Baumes, R. L., & Cordonnier, R. (1986). Stability of free and bound fractions of some aroma components of grapes cv. Muscat during wine processing, preliminary results. *American Journal of Enology and Viticulture, 37*, 112–114.

Gunata, Y. Z., Bitteur, S., Brillouet, J. M., Bayonove, C. L., & Cordonnier, R. (1988). Sequential enzymatic hydrolysis of potentially aromatic glycosides from grape. *Carbohydrate Research, 184*, 139–149.

Gunata, Y. Z, Bayonove, C. L., Tapiero, C., & Cordonnier, R. (1990). Hydrolysis of grape monoterpenil β-D-glucosides by various β-glucosidases. *Journal of Agricultural of Food Chemistry, 38*, 1232–1236.

Hernández, L. F., Espinosa, J. C., Fernández-González, M., & Briones, A. (2002). β-glucosidase activity in a *Saccharomyces cerevisiae* strain. *International Journal of Food Microbiology, 80*, 171–176.

Iland, P. G., Cynkar, W., Francis, I. L., Williams, P. J., & Coombe, B. G. (1996). *Australian Journal of Grape and Wine Research, 2*, 171–178.

Lao, C., López Tamanes, E., Lamuela Raventos, R. M., Buxaderas, S., & Torre Boronat, M. (1997). Pectic enzyme treatment effects on quality of white grape musts and wines. *Journal of Food Science, 62*, 1142–1144.

Manzanares, P., Rojas, V., Genovés, S., & Vallés, S. (2000). A preliminary search for anthocyanin-β-D-glucosidase activity in non-*Saccharomyces* wine yeasts. *International Journal of Food Science and Technology, 35*, 95–103.

McCloskey, L. P., Sylvan, M., & Arrhenius, S. P. (1996). Descriptive analysis for wine quality experts determining appellations by Chardonnay wine aroma. *Journal of Sensory Studies, 11*, 49–67.

Mendes-Ferreira, A., Clímaco, M. C., & Mendes Faia, A. (2001). The role of non-*Saccharomyces* species in realising glycosidic fraction of grape aroma components- a preliminary study. *Journal of Applied Microbiology, 91*, 67–71.

Nicolini, G., Versini, G., & Adalla, Serra (1993). Exogenous pectolytic-glycosidase enzymes in musts and wines: analytical and sensory aspects. *Enotecnico, 29*, 55–68.

Razungles, A., Gunata, Z., Pinatel, S., Baumes, R. Y., & Rayonove, C. (1993). Quantitative studies on terpenes, norisoprenoids and their precursors in several varieties of grapes. *Sciences des Aliments, 13*, 59–72.

Reyero, R., Garijo, J., Díaz Plaza, E., Cuartero, H., Salinas, M. R., & Pard, F. (2000). Comparison of aroma composition of six monovarietal red wines. *Alimentación, Equipos y Tecnología, 19*, 101–110.

Reynolds, A. G., & Wardle, D. A. (1997). Flavour development in the vineyard: impact of viticultural practices on grape monoterpenes and their relationship to wine sensory response. *South African Journal for Enology and Viticulture, 18*, 3–18.

Ribereau-Gayon, P., Glories, Y., Maujena, A., & Dubourdieu, D. . *Handbook of enology. The chemistry of wine and stabilization and treatments* (Vol. 2). New York: Wiley.

Strauss, M. L. A., Jolly, N. P., Lambrechts, M. G., & van Rensburg, P. (2001). Screening for the production of extracellular hydrolytic enzymes by non-*Saccharomyces* wine yeasts. *Journal of Applied Microbiology, 91*, 182–190.

Tingle, M. Y., & Halvorson, H. (1971). A comparison of β-glucanase and β-glucosidase in *S. lactis*. *Biochimica et Biophisica Acta, 250*, 165–171.

Voirin, S., Baumes, R., Bitteur, S., Gunata, Z., & Bayonove, C. (1990). Novel monoterpene disaccharide glycosides of *Vitis vinifera* grapes. *Journal of Agricultural and Food Chemistry, 38*, 1373–1378.

Voirin, S. G., Baumes, R. L., Gunata, Z. Y., Bitteur, S. M., & Bayanove, C. L. (1992). Analytical methods for monoterpene glycosides in grape and wine I. XAD-2 extraction and gas chromatographic-mass spectrometric determination of synthetic glycosides. *Journal of Chromatogric, 590*, 313–328.

Voirin, S. G., Baumes, R. L., Sapis, J. C., & Bayanove, C. L. (1992). Analytical methods for monoterpene glycosides in grape and wine II. Qualitative and quantitative determination of monoterpene glycosides in grape. *Journal of Chromatography, 595*, 269–281.

Williams, P. J., Strauss, C. R., Wilson, B., & Massy-Westropp, R. A. (1982). Studies on the hydrolysis of *Vitis vinifera* monoterpene precursor compounds and model monoterpene β-D-glucosides

rationalizing the monoterpene composition of grapes. *Journal of Agriculture and Food Chemistry, 30*, 1219–1223.

Williams, P., Cynkar, W., Francis, I., Gray, J., Iland, P., & Coombe, B. (1995). Quantification of glycosides in grapes, juices, and wines through a determination of glycosyl glucose. *Journal of Agricultural and Food Chemistry, 43*, 121–128.

Zoecklein, B. W., Marcy, J. E., Williams, J. M., & Jasinski, Y. (1997). Effect of native yeasts and selected strains of *Saccharomyces cerevisiae* in glycosyl glucose, potential volatile terpenes, and selected aglycones of White Riesling (*Vitis vinifera* L.) wines. *Journal of Food Composition and Analysis, 10*, 55–65.