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Analysis of red grape glycosidic aroma precursors by glycosyl glucose quantification

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ABSTRACT

A method has been developed to analyse the glycosidic aroma precursor of non aromatic red grapes in terms of glucose GG. Due to the matrix interferences, an extract free of glycosylated polyphenols (especially anthocyanins and flavonols) and free glucose has been prepared. Such interferences have been eliminated by combining the use of Oasis MCX SPE and Fehling reagent. The glycosyl aroma precursor's fraction was subjected to an acidic hydrolysis (pH 1, 100 °C, 1 h), where equimolecular proportions of glucose (glucose GG) were released from their respective aglycones and quantified HPLC–IR. Compared with methods that require detailed analysis of the volatile aglycones, this one is able to estimate with good reproducibility the potential aroma of grapes by the only measurement of glucose GG.

1. Introduction

It is known that among all the constituents of wine aroma, the ones from grapes play an important role in determining quality and authenticity. All the compounds associated with grape aroma belong to two groups of substances that are generated during the secondary metabolism: free volatiles or odorant molecules, and aroma precursors or odourless molecules that under certain conditions can be transformed into odorants. In the latter group of substances, the most abundant and the ones that participate more actively on the aroma of wine are the glycosylated precursors and for this reason, they are known as grape aroma potential. Knowledge of the aromatic potential would allow us to optimize some steps in the winemaking process (maceration time, enzyme addition, etc.) in order to obtain high quality wines and could even lead to new forms of carrying out the winemaking process. This is why there is a demand in viticulture for a simple analytical method to determine the contribution of these compounds.

The important role of glycosylated secondary metabolites as aroma precursors in fruits, especially grapes, has stimulated the development of analytical methods for their quantification [1,2]. Of special importance are the studies done by Iland et al. [3–5]. This is an important scientific goal since the individual analysis of aroma precursors is quite complex due to their chemical and physical properties and to the trace amounts found in grapes, especially in non-aromatic varieties which are the majority of winemaking grapes [1]. These precursors consist of a volatile aglycone linked to one molecule of glucose by O-glycosidic link which can be broken at low pH [6–8] or by the action of glycosidase enzymes [7,9–11]. Enzymatic hydrolysis is specific to certain aglycones, whereas acid hydrolysis is not selective, therefore allowing the release of all volatile aglycones. The potential aroma of grapes can then be estimated by measuring the volatile aglycones or the glycosidic glucose (glucose GG) which are released at equimolecular proportions.

The analysis of volatile aglycones requires a volatile fractioning on Amberlite resins [12] through a C_{18} cartridge [4], or on Lichrolut EN polymeric resins [6], which are then eluted using different solvents and concentrated for later gas chromatography–mass spectrometry analysis [6,7,12,13]; whereas the analysis of glucose GG seems to be more simple, using UV–vis spectroscopy or high performance liquid chromatography [4,5,14,15,17]. Nevertheless, we should bear in mind that when the glucose GG analysis option is chosen, other free glucose present in the fruit or glucose from other glycosylated compounds may interfere with the analysis. Among the group of glycosylated secondary metabolites, polyphenolic compounds are heavily involved in the colour of the grapes and wine, but not in the aroma. Especially anthocyanins are the most abundant group in red grapes, being responsible for the red and blue colours. Also, red and white grapes have flavonols, which are another group of polyphenol glycosides whose concentrations exceed most of the grape glycosidic aroma precursors. Therefore, it is necessary to eliminate all these interferences before breaking down the O-glycosidic bond to release glucose GG from the aroma precursors.

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The purpose of this study was to develop a method for the determination of glycosidic aroma precursors in red grapes by analysing the glucose released by acid hydrolysis of the precursors, with previous elimination of the free glucose and glycosylated polyphenol grape interferences. The goal of this study is to apply the method in a non aromatic red variety, like Bobal, where the target compounds concentration is low.

2. Experimental

2.1. Grapes and their extract

The grapes used were of the red Bobal variety collected during 2010 harvest, under proper sanitary conditions, and harvested at the optimal stage of maturity in three different areas of Castilla-La Mancha (Spain) (Bobal 1, Bobal 2, Bobal 3).

2.2. Grapes extract preparation

From a selection of one hundred berries, 50 g of grapes were separated and 50 g of a 50:50 (v/v) absolute ethanol/water solution was added. The mixture was crushed for 30 s (without breaking seeds) with a Robot Coupe GT 550 (Switzerland) at 1400 rpm, and macerated in a closed flask for 2 h at 25 °C. All extract volumes have been adjusted to 70 mL with the same ethanolic solution (50%, v/v), centrifuged at 4000 rpm for 10 min and kept for later analysis. Extracts were prepared by triplicate.

2.3. Glycosyl-glucose (glucose GG) determination

Due to the complex composition of grape extracts and the interferences produce in the determination of glucose GG, each of the procedure steps prior its quantification will be assayed by HPLC coupled to a refractive index detector (RID).

2.3.1. Elimination of glycosidic phenolic compounds interferences by solid phase extraction (SPE)

1.97 mL of a glycosyl aroma reference standard (GARS) together with 30 μL of HCl pH 1 were added to 2 mL of sample. The GARS, a 20 mM β-D-phenylglucose solution, was prepared in ethanol/water at 50% (v/v) (Sigma–Aldrich, Barcelona, Spain). The Oasis MCX SPE cartridge of 60 μm (6 cc–500 mg) (Waters Corp., Milford, MA, USA) was previously conditioned with 5 mL of methanol, 5 mL of Milli-Q water, 5 mL of HCl pH 1 and finally 5 mL of Milli-Q water. Once conditioned, the sample (4 mL) was then passed through the Oasis cartridge with a 2.5 mL/min flow rate till the eluate (~3.5 mL) was colourless. In this case, Bobal colour compounds (mainly anthocyanins) are retained after passing through two cartridges. Sample washing is not necessary as the colourless eluate will already have the glycosidic aroma precursors; then the fraction that is studied is the one first eluted. Each extract was assayed in duplicate.

The reference standard content, chosen for its structural similarities with a glycosidic aroma precursor, was used to determine the compound retention factor into the cartridge and it will be used to correct the final results expression.

The same procedure was carried out with the homogenate sample (2 mL) to which 1.97 mL of a 50:50 (v/v) absolute ethanol/water solution was added, so the hydrolysis step of the standard will not interfere with the measurement.

2.3.2. Removal of free glucose

The elimination of free glucose in the eluates, with and without the reference standard, was performed by reaction with Fehling's reagent (Panreac, Spain). To this end, 1 mL of Fehling A and 1 mL of Fehling B were added to 1 mL of the previous sample (colourless eluate) and placed in a water bath at 70 °C for 30 min. After sample tempering, 0.2 g of $CaCl_2$ (Panreac, Spain) was added, shaken vigorously at 1800 rpm (Vortex shaker), and cooled down to 0 °C in a water bath and finally centrifuged at 4000 rpm for 10 min. The supernatant was then filtered through a PTFE filter (0.45 μm, 13 mm diameter, Millipore).

2.3.3. Hydrolysis of glycosidic aroma precursors (GAP)

The previous supernatants (with and without GARS) were acidified by adding 0.2 mL of HCl to pH 1 per 0.8 mL of sample. Hydrolysis took place in a heating block at 100 °C for 1 h. Each supernatant was assayed in duplicate.

The hydrolysis factor was estimated as the ratio between the concentration of the glycosidic aroma reference standard (GARS) before and after hydrolysis.

2.3.4. Glucose determination by HPLC–RID

Glucose analysis was carried out by high performance liquid chromatography coupled to a refractive index detector (Agilent 1100, Palo Alto, CA, USA). The chromatographic column was a PL Hi-Plex H, 8 μm, 300 mm × 7.7 mm (Varian, Middelburg, Holland). The solvent used was H_2SO_4 (0.004 M) at a flow rate of 0.4 mL/min at 75 °C. The injection volume was 10 μL. The detector was kept at 55 °C and the analysis time was 45 min. Under these conditions, the compounds were eluted as tartaric acid (16.3 min), glucose (17.1 min), fructose (18.3 min), β-D-phenylglucose (29.0 min) and ethanol (35.0 min). Compound identification was carried out with the respective standards (Sigma–Aldrich, Barcelona, Spain). Glucose was dissolved in 100% water whereas the β-D-phenylglucose standard solution was prepared in ethanol/water (50%, v/v). Quantification of glucose (GG) and β-D-phenylglucose was carried out from their respective calibration curves (at concentrations ranging between 0.25 and 35 mM for both compounds ($R^2 > 0.99$).

2.4. Expression of results

The results are expressed in mmol of glucose GG/kg of grapes. Besides the dilution factor, other corrections that are carried out to express the final results are: (a) retention rate in the SPE cartridge of the aroma precursors (it is assumed that the GARS is added to the sample); (b) percentage of hydrolysis of aroma precursors (assuming that GARS is added to the sample). Then,

$$\text{GAP (concentration mmol of glycosidic aroma precursors)/kg of grape} = [\text{GG}] \times \text{HF} \times \text{DF} \times \text{CRF} \times F_{\text{ext}} \times \text{FU}$$

where [GG]: glucose concentration of the hydrolysed sample (mM) given by HPLC; (HF) hydrolysis factor: ratio between the concentration of the GARS and the difference in concentration before and after hydrolysis measured by HPLC. In all cases HF is 1, as the samples are completely hydrolysed; (CRF) cartridge retention factor: ratio between the concentration of the GARS before and after passing the SPE cartridge, measured by HPLC; (DF) dilution factor is 7.5. F_{ext}: dilution factor of the extract which is 2; (FU) factor of grape mass: ratio between the volume (L) of the sample strain and the initial grape mass (50×10^{-3} kg).

2.5. Study of the precision of the method

Direct determination of the precision of aroma precursor extraction cannot be performed individually as it is an indirect determination of the precursors depending on glucose release during acid hydrolysis. Estimated accuracy and precision of the glucose GG method were determined for a Bobal grape extract where a given concentration of β-D-phenylglucose was added; and in parallel, a standard solution β-D-phenylglucose was also analysed. To

further assess the precision of the protocol, 3 replicates of each Bobal grape extract were prepared and analysed in triplicate ($n = 9$), and the mean and coefficient of variation were calculated from these results.

Quantification (LOQ) and detection limits (LOD) of glucose GG determination have been calculated as 8 and 3 times the signal to noise ratio, measured by HPLC–RID.

3. Results and discussion

The proposed method is based on the quantification of glucosyl-glucose (glucose GG) released from the aroma glycosidic precursors, of a red variety, by acid hydrolysis. Taking as reference the published methods which are still used today for evaluating the glucose GG content in white and red grapes [4,5,15,17]. In general, these methods perform an extraction of all glycosylated compounds (polyphenols and aroma) that are then eluted and hydrolysed in an acid medium. Total glucose GG is determined with enzymatic reagents for UV–vis spectroscopy, and glucose GG aroma precursors are calculated by subtracting the total glucose GG content, the content of anthocyanins and other phenolic compounds that were first assayed by Zoecklein et al. [17]. The same authors indicate the need to improve these methods by carrying out a selective separation of polyphenolic and aroma glycosides, and by searching for a glycosyl aroma standard to use as reference in the different analytical steps, as addressed in this study.

The point of using a non aromatic variety such as Bobal is because most studies on glycosyl aroma precursors are carried out in white aromatic varieties, which have a high glycosyl aroma precursors concentration and with no anthocyanins interferences, resulting in a less complex method. Bobal variety is also much extended around the world, especially in the centre-east of Spain, and it is well known for its colour contribution but few studies are found on its aroma characteristics.

3.1. Optimization of the method

Due to the diversity of commercial sugar enzymatic kits and their limitations on the amount of glucose that can be measured, in the proposed method the glucose test is performed by HPLC technique using a refractive index detector. At the same time, it is possible to detect and determine in a single analysis both the glucose and the glycosidic aroma reference standards (GARS) selected, along with other compounds present in grapes (tartaric acid and fructose), or added to the samples during the preparation of the extract (e.g. ethanol) (Fig. 1). This is also one of the reasons why β-D-phenylglucose was selected as the reference glycosidic aroma standard, as other standards such as *n*-octyl glucose used by other authors [14,15] were not accurately detected by HPLC and have an erratic behaviour in OASIS resins. β-D-Phenylglucose standard was also selected by other authors when assayed the oligosaccharides and other glycosides in wines [16].

As already mentioned, one of the problems found with the existing methods, especially when red varieties are assayed [5,17],

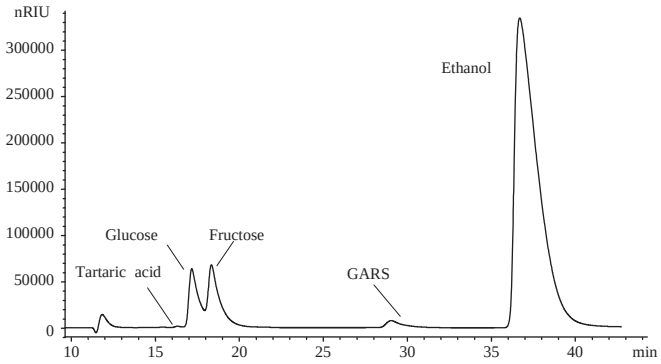

Fig. 1. HPLC chromatogram of an ethanolic Bobal extract.

is the impossibility of separating polyphenolic and aroma glycosides. Currently, the market provides a wide variety of extraction cartridges, and among them, the Oasis MCX cartridges allow the selective separation of glycosylated components, as anthocyanins and other phenolic compounds are retained within the cartridge [18,20]. It is worth mentioned that SPE cartridge can be reused after 5 times [19]. For extraction of flavonols it is necessary to use methanol, whilst the extraction of anthocyanins requires ammonia and methanol. In a first stage, the retention percentage of the phenolic compounds within the SPE cartridge was studied using GARS with a structure similar to a glycosidic aroma precursor. A preliminary test conducted in an ethanolic solution to avoid interferences with other glycosidic aromatic compounds revealed that only 14.5% of the reference standard was retained, thus facilitating the use of the Oasis SPE cartridge (Table 1). Bobal grape extract matrix effect was studied in relation to GARS in order to follow its retention rate on the SPE cartridge by using three different Bobal grape samples, being each extract analysed six times, making an average values of $n = 18$. The eluate obtained still preserved some of its colour, indicating the presence of anthocyanins, a result which was then corroborated by an HPLC–DAD analysis method [21] at 520, 360 and 280 nm. The eluate obtained was passed through a second cartridge, and this time the eluate was colourless, thus confirming the absence of anthocyanins (520 nm), although a small proportion of flavonols (360 nm) that were eliminated in the later stages were still found. In relation to GARS within the grape extract, we observed an important matrix effect with a cartridge retention factor range between 2.80 against the 1.17 factor found in the ethanolic solution (Table 1), for this reason it is necessary to test the retention behaviour of GARS in every matrix analysed. In all cases, good coefficients of variation of cartridge retention factor (CRF) mean values were observed, as for GARS standard solution is 1% and for Bobal grape extract is about 9%.

Once the anthocyanins were removed from the extract, other important point that needed solving was about the interferences produced by the free glucose present in the grapes, as can be seen in Fig. 2, where the eluate also contained tartaric acid, fructose and glucose. The removal of free glucose was performed with the

Table 1
β-D-phenylglucose standard Oasis MCX cartridge retention factor within an standard solution and Bobal grape extract.

	Standard solution[a,b]		Bobal grape extract[c]	
	[GARS] (mM)	σ	[GARS] (mM)	σ
Before SPE cartridge	10.8	0.3	10.7	0.2
After SPE cartridge	9.3	0.2	3.9	0.6
Average cartridge retention factor (CRF)	1.17 ± 0.01	2.8 ± 0.3		

[a] Standard solution prepared with 2 mL of β-D-phenylglucose (20 mM), 1.97 mL of a 50% (v/v) ethanol/water solution and 0.03 mL of HCl pH 1.
[b] $n = 6$.
[c] $n = 18$.

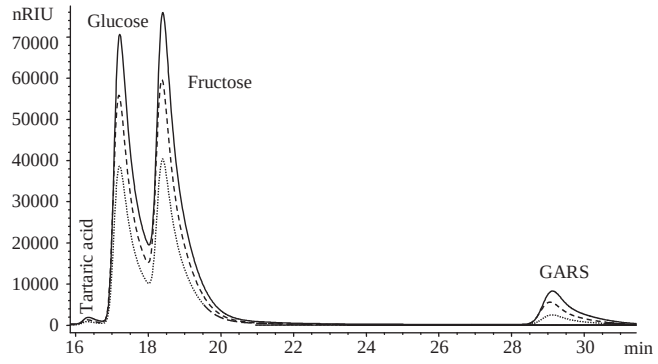

Fig. 2. HPLC chromatogram of a Bobal extract before (—) and after (—) passing the first Oasis SPE cartridge and passing the second Oasis SPE cartridge (. . .).

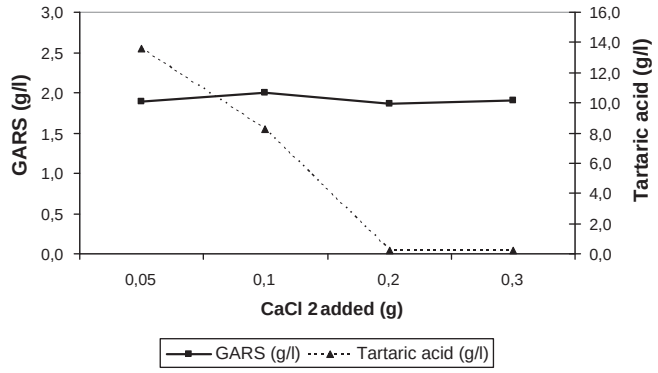

Fig. 3. Effect of $CaCl_2$ on the concentration of tartaric acid and GARS.

Fehling reagent which eliminated the glucose and fructose in the sample but increased the concentration of tartaric acid, since it is one of the constituents of Fehling. It was also necessary to reduce the content of tartaric acid to avoid overlapping with the peak of glucose GG, which was achieved by its precipitation with $CaCl_2$. Different proportions were tested (0.05, 0.2, 0.3 and 0.5 g) of $CaCl_2$ in 3 mL of the sample (1 mL of the eluate + 1 mL of Fehling A + 1 mL of Fehling B). The analysis of tartaric acid showed that tartaric precipitation did not affect the reference standard and, with the addition of 0.2 g of $CaCl_2$, it was possible to decrease tartaric acid to concentrations that did not interfere in the determination of glucose GG (Fig. 3). The samples in which free glucose was removed and the tartaric acid content reduced were also analysed by HPLC–DAD to check the flavonol content. Chromatograms were obtained with a total absence of these compounds, thus ensuring they contained no glycosylated polyphenol that could interfere with the subsequent hydrolysis process.

Once the free glucose and the interferences with tartaric acid were eliminated and the glycosyl aroma precursor fraction isolated, it was necessary to study the hydrolysis time. First we submitted the ethanolic solution of the reference standard, after being subjected to the previous steps, to 0, 1/2, 1, 2, 4, 12 and 24 h of hydrolysis time. Fig. 4 shows that before hydrolysis GARS is the predominant compound in the sample, no free glucose is detected and the area of tartaric acid does not mask the glucose. After

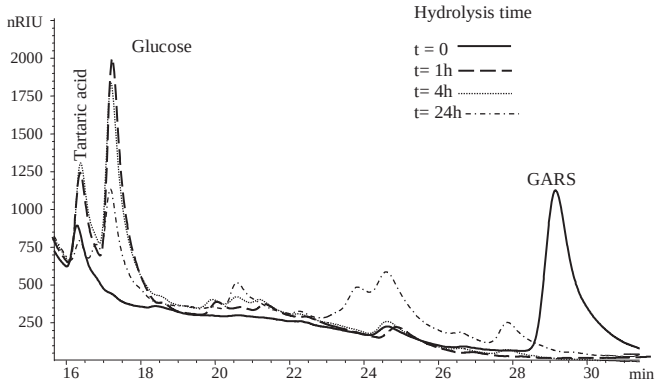

Fig. 4. HPLC chromatograms of the hydrolysed eluate from GARS solution which has been previously treated with Fehling reagent and $CaCl_2$, and submitted to different hydrolysis times (0, 1, 4 and 24 h).

Table 2
Concentration of glucose GG (mM) in samples with and without GARS and its recovery percentage ($n = 12$).

Sample	Without GARS [mM]	With GARS [mM]	Recovery (%)
Bobal 1	2.22 ± 0.05	12.1 ± 0.8	92.3 ± 7.5
Bobal 2	2.43 ± 0.08	13.3 ± 0.4	101.9 ± 4.7
Bobal 3	2.41 ± 0.06	14.5 ± 1.2	113.4 ± 11.1
Average	2.4 ± 0.1	13.3 ± 1.3	102.6 ± 10.6

1 h GARS was not detected and the concentration of glucose GG reached the highest level, whilst longer hydrolysis time decreased glucose GG level. When hydrolysis was performed with Bobal grape extracts together with the reference standard, the same results were observed i.e. after 1 h the reference standard was not detected and the glucose GG content was reached its highest concentration.

To avoid the interference of the glucose released from GARS, the grape extracts were also analysed changing the GARS by the same quantity of the ethanolic solution used as extractant.

3.2. Precision, quantification and detection limits of the method

The analytical step used to obtain the percentage of recovery for each sample is immediate post-hydrolysis, i.e. the samples with and without reference standard have gone through the stages of extraction in the SPE cartridge, along with treatments with Fehling, $CaCl_2$, and acid hydrolysis at 100 °C for 1 h. The results show recovery rates ranging between 92.29% and 113.44% (Table 2), suitable for the method's objective. Low variation coefficients were found in all cases, which demonstrate the adequate method reproducibility (Table 2).

The detection (LOD) and quantification (LOQ) limits of glucose analysed in the HPLC–RID were 16.39 mg/L and 21.13 mg/L, respectively. Within the samples, the LOD and LOQ of glucose GG per kg of grape were 2.51 mmol and 5.01 mmol, respectively.

3.3. Determination of glycosidic aroma precursors by glucose GG analysis

The method was applied to different samples of Bobal grapes from three different areas in Castilla-La Mancha (Spain). From each

Table 3
Concentration of glycosyl aroma precursors of different Bobal samples expressed as concentration of glucose GG (mmol) per kg of grape ($n = 12$).

No. of extractions	Bobal 1	σ	Bobal 2	σ	Bobal 3	σ
E1	5.4	0.1	6.9	0.4	6.22	0.04
E2	7.1	0.2	7.1	0.5	6.1	0.2
E3	7.0	0.1	9.4	0.4	6.5	0.2
Average	6.5 ± 0.8		7.8 ± 1.2		6.5 ± 0.6	

sample three different extracts were prepared and each extract was applied in duplicate using the complete analytical protocol detailed in Section 2. Table 3 shows the contents of glycosylated aroma precursors in each extract, expressed as mmol glucose GG concentration per kg of grapes, where Bobal 2 has a higher aroma potential than the other two samples analysed. Very low variation coefficients were found in all cases.

4. Conclusions

A method for the analysis of glycosidic aroma precursors in red grapes has been developed based on HPLC–RID determination of glucose released by acid hydrolysis. The interferences produced within the red viniferas due to glycosidic polyphenols and free glucose was resolved by using an Oasis SPE cartridge and Fehling reagent, respectively.

Cartridge retention factor of the glycosidic aroma precursors and the percentage of hydrolysis were calculated by using a glycosyl aroma reference standard (GARS), β-D-phenylglucose, due to its structural similarity with glycosidic aroma precursors as well as its capacity to be analysed by the same HPLC method as the glucose GG.

Compared to methods that require detailed analysis of the volatile aglycones, the proposed one has the advantage of being able to estimate the aroma potential of the grapes with only the glucose GG measurement, showing at the same time a high reproducibility. As glucose GG analysis is carried out on an extract free of interferences, other conventional analytical methods available by wineries, different from HPLC, can be used.

The simplicity of the method makes it useful in viticulture, as it will reveal the impact of the vineyard treatments on the aroma potential of grapes by using a single analytical parameter, in the same way phenolic maturation analysis is carried out at the moment.

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References

[1] C. Bayonove, Z. Günata, J.C. Sapis, R. Baumes, I. Dugelay, C. Grassin, Rev. Œnol. 64 (1992) 165–169.
[2] Z. Günata, I. Dugelay, J.C. Sapis, R. Baumes, C. Bayonove, in: P. Scherier, P. Winterhalter (Eds.), Flavour Precursor Studies, Allured Publish. Corp., USA, 1993, pp. 219–234.
[3] N.A. Abbott, P.J. Williams, B.G. Coombe, in: C.S. Stockley, R.S. Johnstone, P.A. Leske, T.H. Lee (Eds.), Proceedings of the Eighth Australian Wine Industry Technical Conference, Winetitles, Adelaide, 1993, pp. 72–75.
[4] P.J. Williams, W. Cynkar, I.L. Francis, J.D. Gray, P.G. Iland, B.G. Coombe, J. Agric. Food Chem. 43 (1995) 121–128.
[5] R.S. Whiton, B.W. Zoecklein, Am. J. Enol. Vitic. 53 (2002) 315–317.
[6] M.J. Ibarz, V. Ferreira, P. Hernández-Orte, N. Loscos, J. Cacho, J. Chromatogr. A 1116 (2006) 217–229.
[7] N. Loscos, P. Hernández-Orte, J. Cacho, V. Ferreira, J. Agric. Food Chem. 57 (2009) 2468–2480.
[8] M. Ugliano, L. Moio, Anal. Chim. Acta 621 (2008) 79–85.
[9] D.J. Caven-Quantrill, A.J. Buglass, J. Chromatogr. A 1117 (2006) 121–131.
[10] M.A. Sefton, I.L. Francis, P.J. Williams, J. Food Sci. 59 (1994) 142–147.
[11] M.A. Sefton, Aust. J. Grape Wine Res. 4 (1998) 30–38.
[12] Z. Günata, C. Bayonove, R. Baumes, R. Cordonnier, J. Chromatogr. A 331 (1985) 83–90.
[13] M.A. Pedroza, A. Zalacain, J.F. Lara, M.R. Salinas, Food Res. Int. 43 (2010) 1003–1008.
[14] M. Arévalo Villena, J. Díez Pérez, J.F. Ubeda, E. Navascués, A.I. Briones, Food Chem. 99 (2006) 183–190.
[15] P.G. Iland, W. Cynkar, I. Francis, P.J. Williams, B.G. Coombe, Aust. J. Grape Wine Res. 2 (1996) 171–178.
[16] A.I. Ruíz-Matute, M.L. Sanz, M.V. Moreno-Arribas, I. Martínez-Castro, J. Chromatogr. A 1216 (2009) 7296–7300.
[17] B.W. Zoecklein, L.S. Douglas, W. Jasinski, Am. J. Enol. Vitic. 51 (2000) 420–423.
[18] N. Castillo-Muñoz, M. Fernández-González, S. Gómez-Alonso, E. García-Romero, I. Hermosín-Gutiérrez, J. Agric. Food Chem. 57 (2009) 7883–7891.
[19] N. Castillo-Muñoz, S. Gómez-Alonso, E. García-Romero, I. Hermosín-Gutiérrez, J. Agric. Food Chem. 55 (2007) 992–1002.
[20] S. González-Manzano, C. Santos-Buelga, J.J. Pérez-Alonso, J. Rivas-Gonzalo, M.T. Escribano-Bailón, J. Agric. Food Chem. 54 (2006) 4326–4332.
[21] D. Cozzolino, M.J. Kwiatkowski, M. Parker, W.U. Cynkar, R.G. Dambergs, M. Gishen, M.J. Herderich, Anal. Chim. Acta 513 (2004) 73–80.