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Analytical Methods

Rapid HPLC analysis of amino acids and biogenic amines in wines during fermentation and evaluation of matrix effect

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ABSTRACT

A rapid HPLC method has been developed for the simultaneous determination of 23 amino acids, 10 biogenic amines and the ammonium ion in wine. Samples were pre-column derivatised with diethyl ethoxymethylenemalonate and separated using reversed-phase HPLC within 30 min. The matrix effect was evaluated when measuring samples taken from different stages of fermentation. Most compounds showed no obvious matrix effect, whereas proline, ethanolamine and spermine had remarkably different responses to variable concentrations of sugar. High concentrations of sugar affected the pH of the derivatisation reaction system; proline, ethanolamine and spermine derivatives were sensitive to this effect. Matrix-matched calibration was used for the quantification of these compounds. Validation of the method showed that it was accurate, reproducible and efficient for the simultaneous determination of amino acids and biogenic amines in wines during fermentation. As a specific application of the method, red wine samples taken from different stages of fermentation were analysed.

1. Introduction

Free amino acids and ammonium are the most important nitrogen compounds in grape must. They are essential to the growth and metabolism of yeast during the course of alcoholic fermentation (Bell & Henschke, 2005). A limitation in their concentration could increase the risk of sluggish or stuck fermentation (Arias-Gil, Garde-Cerdán, & Ancín-Azpilicueta, 2007; Bell & Henschke, 2005). On the other hand, wines with high concentrations of residual amino acids have a greater risk of microbiological instability with the possible formation of biogenic amines and ethyl carbamate, which have a negative impact on wine quality (Garde-Cerdán et al., 2011; Uthurry, Suárez Lepe, Lombardero, & García Del Hierro, 2006). Amino acids are also precursors of some volatile compounds, such as higher alcohols, aldehydes, esters and ketonic acids, which make up the majority of wine aroma (Callejón, Troncoso, & Morales, 2010; Soufleros, Bouloumpasi, Tsarchopoulos, & Biliaderis, 2003; Torrea et al., 2011; Valero, Millán, Ortega, & Mauricio, 2003). Moreover, the composition of amino acids in must and wine has been used as marker of variety, vintage and geographical origin of grapes used to produce wines (Garde-Cerdán et al., 2009; Soufleros et al., 2003).

Biogenic amines are derived mainly from the decarboxylation of their precursor amino acids. They may be formed by the action of yeast, lactic acid bacteria or other contaminating microorganisms during alcoholic and malolactic fermentation (Lonvaud-Funel, 2001). Some biogenic amines are already present in must (Del Prete, Costantini, Cecchini, Morassut, & Garcia-Moruno, 2009; Herbert, Cabrita, Ratola, Laureano, & Alves, 2005; Manfroi, Silva, Rizzon, Sabaini, & Glória, 2009; Peña-Gallego, Hernández-Orte, Cacho, & Ferreira, 2012). The most commonly found amines in wine are histamine, tyramine, putrescine and cadaverine. Some of these amines, such as histamine and tyramine, are harmful to human health (Anli & Bayram, 2008; Hernández-Orte, Peña-Gallego, Ibarz, Cacho, & Ferreira, 2006). In the case of histamine, some countries have recommended maximum limits in wine (Hernández-Orte et al., 2006; Izquierdo Cañas, García Romero, Gómez Alonso, Fernández González, & Palop Herreros, 2008; Proestos, Loukatos, & Komaitis, 2008).

For these reasons, amino acids and biogenic amines are important for the quality control of wine. It is necessary to develop a more accurate and efficient determination method of these compounds. HPLC is by far the most frequently reported technique for the determination of these compounds in foods (Mandrioli, Mercolini, & Raggi, 2013; Önal, 2007). The direct analysis of these two families of compounds is difficult due to their different

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structures and the absence of a specific chromophore; therefore pre- or post-column derivatisation is usually needed. Typical derivatisation reagents include dansyl chloride, which yields derivatives detectable by fluorescence and ultraviolet spectroscopy (Proestos et al., 2008; Romero, Sánchez-Viñas, Gázquez, & Bagur, 2002), phenylisothiocyanate (PITC) (Palacios, Valcárcel, Caro, & Pérez, 2002) and diethyl ethoxymethylenemalonate (DEEMM) (Alaiz, Navarro, Girón, & Vioque, 1992; Gómez-Alonso, Hermosín-Gutiérrez, & García-Romero, 2007; Redruello et al., 2013), which yield derivatives detectable by ultraviolet spectroscopy, *o*-phthaldialdehyde (OPA) (Kelly, Blaise, & Larroque, 2010; Pereira, Pontes, Câmara, & Marques, 2008), 9-fluorenylmethylchloroformate (FMOC) (Fabiani, Versari, Parpinello, Castellari, & Galassi, 2002) and 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) (Bosch, Alegría, & Farré, 2006; Hernández-Orte et al., 2006), which yield fluorescent derivatives.

DEEMM is a pre-column derivatisation agent. It can react with both primary and secondary amino acids. The derivatisation is quantitative, easy and clean (no side-reaction products), and the derivatives are stable for several days at room temperature. Moreover, the derivatives can be detected with a UV detector, which is available in most laboratories (Alaiz et al., 1992; Gómez-Alonso et al., 2007; Montevecchi, Masino, Chinnici, & Antonelli, 2010). The detection limits, which are below 0.4 mg/L for amino acids and 0.06 mg/L for biogenic amines (Gómez-Alonso et al., 2007), are higher than some methods with fluorescence detection (Arrieta & Prats-Moya, 2012; Callejón et al., 2010; Hernández-Orte et al., 2006), but they are still sufficient for amino acid and biogenic amine analysis in must and wine (Arrieta & Prats-Moya, 2012; Bell & Henschke, 2005; Del Prete et al., 2009; Izquierdo Cañas et al., 2008; Pereira et al., 2008; Peña-Gallego et al., 2012; Soufleros et al., 2003).

The DEEMM derivatisation method has been applied to wines after fermentation (Gómez-Alonso et al., 2007). However, for better quality control of wine, it is essential to monitor the nitrogen status in must, the fermentation trends and the accumulation of biogenic amines during the fermentation process. When using the method in samples taken from different stages of fermentation, the influence of the complex must and wine matrices on the derivatisation reaction and analyte signals must be taken into consideration. Little attention has been paid to the matrix influence on the analysis of amino acids and amines in foods. When the PITC derivatisation method was employed in the determination of amino acids in wine, no matrix effect was observed; however, when the same method was applied to must, a sugar concentration of 210 g/L in must diminished the amino acid signal by approximately 60% and solutions with sugar content below 50 g/L were free from this interference (Orte, Guitart, & Cacho, 1997). Bosch et al. (2006) have applied AQC derivatisation method to the determination of amino acids in milk-cereal based infant food, and they reported that amino acids determination was free from matrix interference. However, also using the AQC derivatisation method, a clear matrix effect was noticed in the case of tyramine and putrescine in must and wine (Hernández-Orte et al., 2006). There is still very limited consideration or evaluation of the matrix influence on DEEMM derivatisation method. Redruello et al. (2013) compared the chromatogram of six cheese samples when using the DEEMM derivatisation method and no matrix effect was observed. However, the matrix of wine changes dramatically during the course of fermentation: the content of sugar generally decreases from about 200 g/L to less than 4 g/L, meanwhile the ethanol content increases from a minimal content to 14–15% (V/V) (Jackson, 2008, chap. 6). So the matrix effect needs to be evaluated for accurate quantification.

The present study developed a reliable and rapid method for the simultaneous analysis of amino acids and biogenic amines in wines during the fermentation process. The elution time was shortened to 30 min. The sensitivity, precision and accuracy suggested that it could be a very valuable tool in monitoring wine quality. Considering the most significant differences between must and wine taken from different stages of fermentation are the contents of residual sugar and alcohol, the matrix effects induced by sugar and alcohol were evaluated thoroughly. Remarkable matrix effect of some amino acid and biogenic amine derivatives was observed in the existence of high content glucose and matrix-matched calibration curve was validated for correcting this effect.

2. Materials and methods

2.1. Reagents

The pure reference compounds and DEEMM were purchased from Sigma (St. Louis, USA), Aldrich (Gillingham, UK) and Fluka (Buchs, Switzerland). HPLC grade acetonitrile and methanol were obtained from Honeywell (New Jersey, USA). Ultrapure water was generated using a Milli-Q purification system (Millipore, USA). Sodium azide was purchased from Sangon (Shanghai, China). Solutions of amino acids and biogenic amines were prepared with 0.1 M HCl.

2.2. Wine samples

Samples from two different stainless steel tanks were taken during the course of alcoholic and malolactic fermentation. The grapes used to make wines were Cabernet Sauvignon from the west region of China in the 2011 harvest.

2.3. Derivatisation

The derivatisation was performed according to the methods of Alaiz et al. (1992) and Gómez-Alonso et al. (2007) with some modifications. The reacting mixture included 430 μL of 1 M borate buffer (pH 9.0), 300 μL methanol, 400 μL sample, 10 μL internal standard (2-aminoadipic acid, 1.00 g/L) and 12 μL DEEMM. The derivatisation reaction was carried out in a screw-cap test tube over 30 min in ultrasound bath. The mixture was then heated at 70–80 °C for 2 h to allow complete degradation of excess DEEMM and other byproducts.

2.4. HPLC analysis

The analyses were performed on an Agilent 1200 series HPLC system. Chromatography separation was performed in an Agilent ZORBAX SB-C18 Rapid Resolution HT column (3.0 mm × 50 mm, 1.8 micron) at 16 °C. The eluent gradient was: 0.6 mL/min flow rate, 6% B for 3 min, then elution from 6% to 14% B in 7 min, kept for 3.5 min, from 14% to 19.5% B in 2 min, from 19.5% to 20% B in 2.5 min, kept for 1 min, from 20% to 26% B in 1 min, from 26% to 30% B in 3 min, from 30% to 50% B in 1 min, from 50% to 63% B in 1 min, kept for 1.5 min, from 63% to 100% B in 1 min, then kept for 1 min, followed by washing and reconditioning the column. The composition of the solvents followed: phase A, 25 mM acetate buffer (pH 5.8) with 0.02% of sodium azide; mobile phase B, 100% acetonitrile. The injection volume was 2 μL. For the detection, a photodiode array detector (G1315D) monitored at 280 nm was used. The target compounds were identified according to the retention times of their corresponding standards. Quantification was performed using the calibration curves of the respective standards, which underwent the same process of derivatisation as the samples (for the quantification of proline, ethanolamine and spermine, matrix-matched

calibration curve was needed). Internal standard method based on the areas of the peaks of the derivatives was used.

2.5. Evaluation of matrix effect

Matrix effect was determined by comparing the peak area of the analytes in different concentrations of sugar or alcohol modelled solutions to that of each analyte obtained in pure water. Five different alcohol concentrations (0%, 3.0%, 6.0%, 9.0% and 12.0%, V/V) and five different glucose concentrations (200.0, 150.0, 100.0, 50.0 and 0 g/L), which represented the process of fermentation, were selected.

2.6. Statistical analysis

All analyses were performed in triplicate. Studies of the correlation coefficient and linear regression, assessment of repeatability, calculation of average, standard deviation and relative standard deviation were performed using Microsoft Excel 2010 software. Significance of analyte response differences among matrices was performed using one-way analysis of variance (ANOVA) test, employing Duncan's multiple range test at significance level $p < 0.05$ (SPSS 17.0 statistical software, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Optimisation of the HPLC analysis

The original method established by Alaiz et al. (1992) could analyse 17 free amino acids after pre-column derivatisation with DEEMM within 40 min. Based on this work, Gómez-Alonso et al. (2007) developed a new method that extended the analytes to 24 amino acids, nine biogenic amines and the ammonium ion in a single injection, while extending the running time to 85 min. In our work, a Rapid Resolution HT analysing column (3.0 mm × 50 mm, 1.8 micron) was used to shorten the analysing time and improve the analysis efficiency. The elution separation program was optimised to separate 23 amino acids, 10 biogenic amines and the ammonium ion simultaneously. Hydroxyproline was not considered in our work because it is not assimilable by yeast and presents in very low amounts in the free form in must and wine (Montevecchi et al., 2010; Taylor, Karunaratne, & Xie, 2012). The gradient program selected allowed adequate separation of all compounds within 30 min. The chromatogram was shown in Fig. 1.

The selected conditions were a compromise between the chromatographic separation of all peaks and the need for a rapid analytical method. This new program could separate as many compounds as the previous method, and the elution time was reduced by half, which was a huge improvement for the efficiency of routine sample analysis. The mobile phase B was changed back to 100% acetonitrile (Alaiz et al., 1992), which could satisfactorily separate all peaks and was easier to get prepared. A wavelength of 280 nm was selected for quantifying because all of the compounds displayed good separation at this wavelength and the intensity of signals were strong enough for quantification.

3.2. Matrix effect

For accurate quantification of the targeted compounds, we evaluated if the matrix interference existed when using the method on

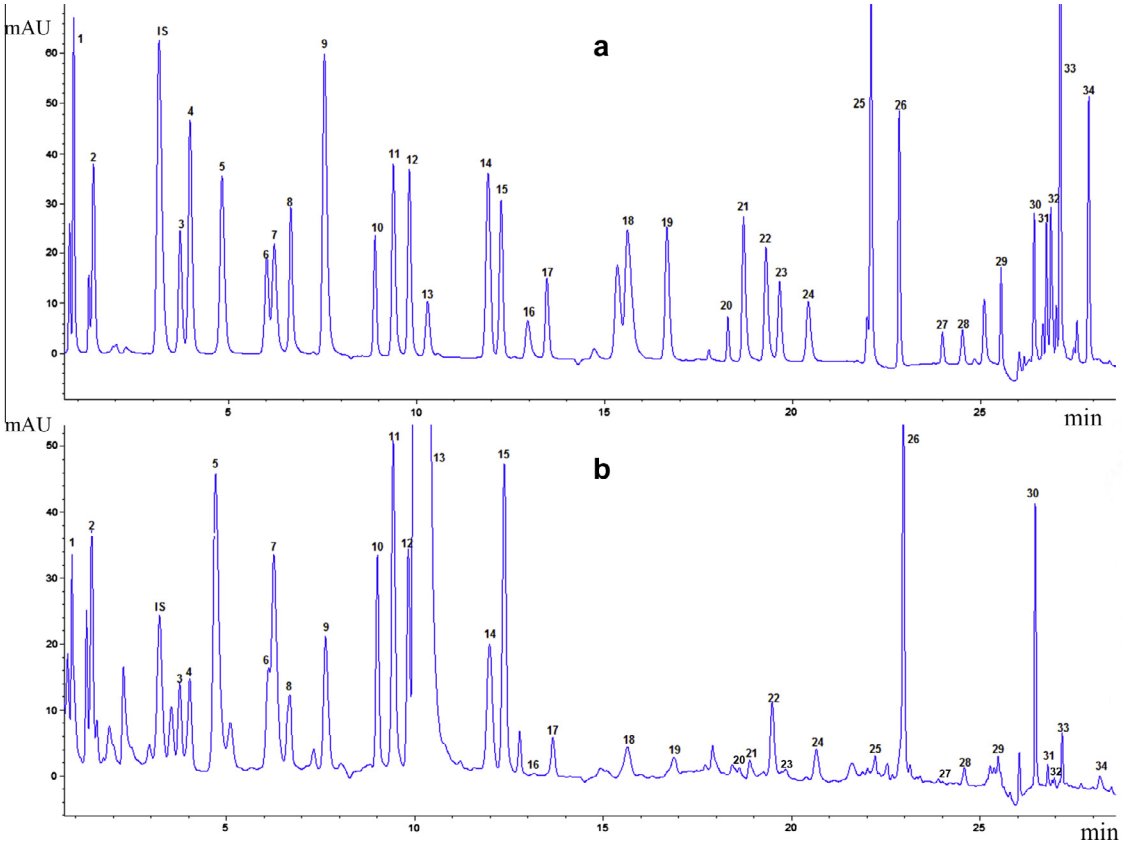

Fig. 1. HPLC chromatograms of the derivatives of amino acids, ammonium ion and biogenic amines at 280 nm, a standard solution (a) and a red wine sample (b). Peak assignments: (1) Asp; (2) Glu; IS, internal standard (L-2-aminoadipic acid); (3) Ser; (4) Asn; (5) Gln; (6) His; (7) Gly; (8) Thr; (9) β-Ala; (10) Arg; (11) Ala; (12) GABA; (13) Pro; (14) NH_4^+; (15) ethanolamine; (16) Tyr; (17) histamine; (18) Val; (19) Met; (20) Cys; (21) Ile; (22) Leu; (23)Trp; (24) Phe; (25) Orn; (26) Lys; (27) agmatine; (28) serotonin; (29) tyramine; (30) putrescine; (31) cadaverine; (32) phenylethylamine; (33) spermidine; (34) spermine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

samples during the fermentation process. Considering the most significant differences between must and wine taken from different stages of fermentation are the contents of residual sugar and alcohol, we estimated these matrix influences on the determination of the targeted compounds. Five different alcohol concentrations (0%, 3.0%, 6.0%, 9.0% and 12.0%, V/V) and five different glucose concentrations (200.0, 150.0, 100.0, 50.0 and 0 g/L), which represented the process of fermentation, were selected for the evaluation of the matrix effect. Standard compounds at the same concentration both in pure water and in the modelled solvents were analysed, and the area ratios (the area of targeted compound to the area of internal standard ratio) in different matrices were compared.

The statistical significance of analyte response difference was determined and the result was shown in Table S1 (Supplementary table). The area ratio differences of proline, ethanolamine and spermine in response to different sugar concentrations were significant at the 0.01 level, and the response difference of methionine, leucine, agmatine and putrescine were significant at the 0.05 level. The area ratio of histidine, proline, tyrosine, spermidine and phenylethylamine showed statistical significant difference at the 0.05 level among different alcohol concentration matrices. Responses of other compounds showed no significant difference. Most compounds' area ratios changed less than 20% in different matrices. Whereas proline, ethanolamine and spermine derivatives showed remarkable matrix effect in the presence of sugar, mostly an inhibition effect (Fig. 2). A sugar concentration above 150.0 g/L diminished their signals by more than 50%. Considering both the statistic analysis result and the changing extent of responses, the three compounds proline, ethanolamine and spermine, responses of which both were significantly different in variable sugar concentration solvents and diminished by more than 50%, were considered to be influenced remarkably by matrix effect. This matrix effect would lead to false quantification results for these compounds if matrix-free standards were used for calibration.

Orte et al. (1997) proposed that using internal standard for quantification could avoid matrix effect, but it did not work under these conditions because the response of the internal standard was nearly the same in different matrices. An influence from the buffer may occur when the complex samples are derivatised, but UV detection does not depend on the buffer type (Rebane & Herodes, 2012). Further experiments were taken to test the pH of the derivatisation reaction system (Table 1). We noticed that the higher the concentration of glucose, the lower the pH of the reaction system (fructose has been tested as well and the data were not shown because it exhibited the same pattern as glucose). The borate buffer failed to maintain the acidity of the reaction system in the presence of too much sugar, whereas the derivatisation needed to occur

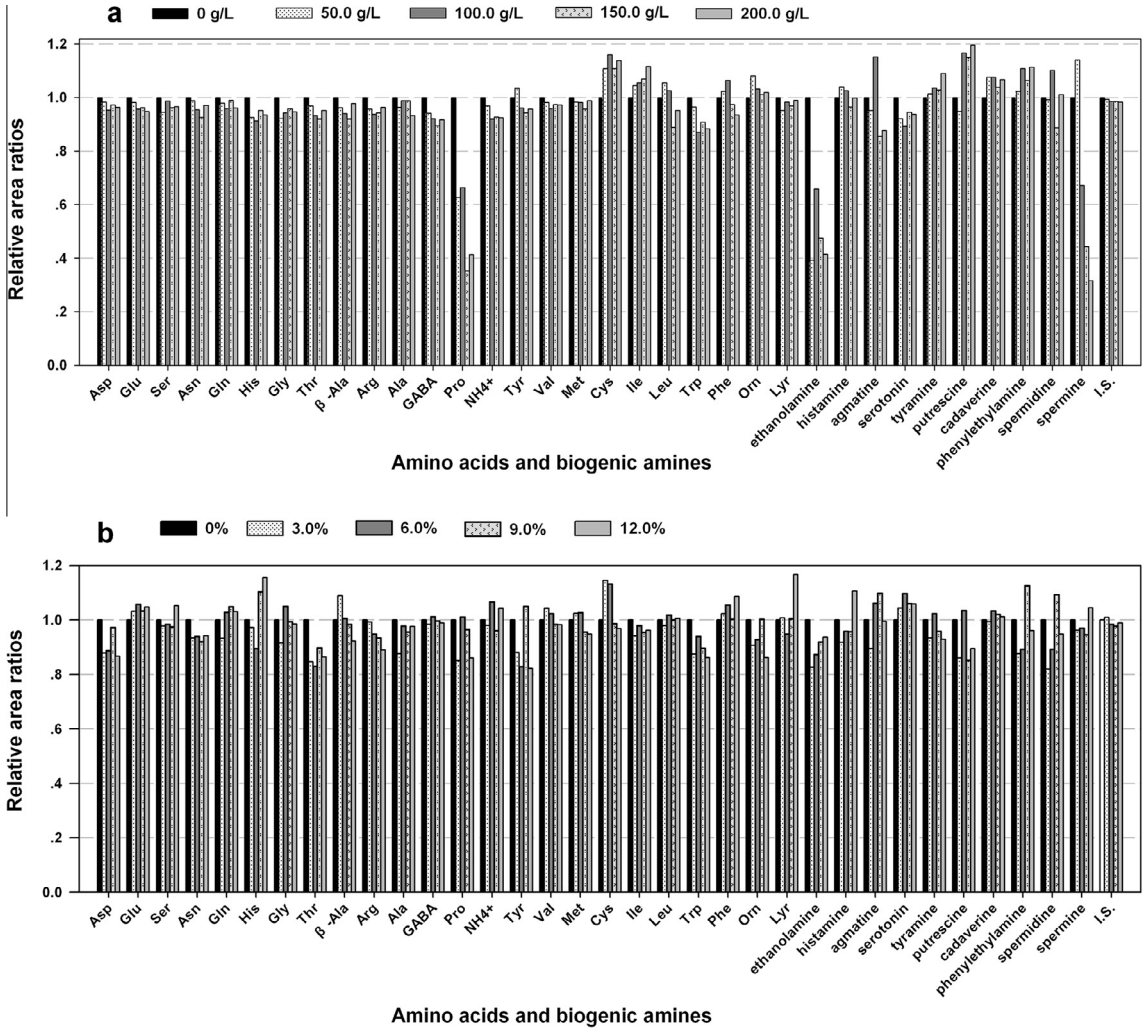

Fig. 2. Responses of standard amino acids and biogenic amines in the presence of various concentrations of residual sugar (a) and ethanol (b). Different responses were presented as relative area ratios, which were calculated as (targeted compound area/internal standard area in different matrices)/(targeted compound area/internal standard area in pure water).

Table 1
pH of the derivatisation reaction system with different matrices (mean ± SD, $n = 3$).

Ethanol concentration (V/V)	pH of reaction system	Glucose concentration (g/L)	pH of reaction system
0%	8.92 ± 0.06	0	8.92 ± 0.06
3.0%	8.75 ± 0.01	50.0	8.34 ± 0.01
6.0%	8.75 ± 0.03	100.0	7.94 ± 0.02
9.0%	8.79 ± 0.02	150.0	7.56 ± 0.02
12.0%	8.80 ± 0.02	200.0	7.34 ± 0.03

at an alkaline system. Proline, ethanolamine and spermine were especially sensitive to pH changes in the reaction system. When samples were prepared with a high sugar concentration, attention must be paid to the influence of the matrix.

Therefore, certain strategies, such as matrix-matched calibration curve, should be used for the reliable quantification. In this study, we chose different concentrations of glucose to model the residual sugar content of must and wine samples, spiked with standards for matrix-matched calibration curve.

3.3. Validation of the method

3.3.1. Linearity, detection limits and repeatability

The linearity, detection limits and repeatability of the method were evaluated using standard samples. Calibration standards were prepared at six concentration points and the concentration ranges were shown in Table 2. All compounds showed good linearity over the tested range with correlation coefficient $R^2 > 0.9930$, except Cys (0.9854). The linearity ranges could satisfactorily quantify must and wine samples. The limits of detection (LODs), which were calculated as a signal to noise ratio of 3, were all below 0.50 mg/L (Phe) for amino acids and 0.10 mg/L (agmatine) for biogenic amines. These LODs were in the same range as the previous method developed by Gómez-Alonso et al. (2007). The within-day precision was determined by analysing the same standard mixture five times within a single day under the same operating conditions (the same method, the same equipments and the same operator) and the between-day precision (intermediate precision) was determined by carrying out the same operation over five independent days. Within-day precision of this method ranged from 0.21% to 3.88% (expressed as the relative standard deviation, RSD) and between-day precision ranged from 0.33% to 8.07%, which showed very good repeatability of the method.

3.3.2. Recovery

Recovery was determined by spiking the musts and wines with amino acids and biogenic amines mixtures (two levels of spiked concentrations were tested, shown in Table 3). Each of the spiked samples was determined in triplicate. Proline, ethanolamine and spermine were quantified using matrix-matched calibration curves, and the remaining compounds were quantified using the matrix-free calibration curves, which used pure water as the solvent. The samples used for the validation of the recovery were taken at days

Table 2
Retention time, calibration variables, sensitivity and precision of the HPLC method for the determination of amino acids and biogenic amines.

Compound	RT/min	Calibration		LODs[a] (mg/L)	Repeatability	
		R^2	Linear range (mg/L)		Within-day[b]	Between-day[c]
Asp	0.875	0.9985	1.00–200.00	0.02	2.16	6.32
Glu	1.400	0.9937	1.00–200.00	0.02	2.37	6.71
Ser	3.705	0.9992	1.00–100.00	0.20	2.65	4.67
Asn	3.968	0.9992	1.00–100.00	0.20	2.75	3.73
Gln	4.817	0.9992	1.00–200.00	0.10	0.46	3.59
His	6.010	0.9990	1.00–200.00	0.20	0.76	4.88
Gly	6.208	0.9982	1.00–100.00	0.20	1.89	5.44
Thr	6.649	0.9992	1.00–200.00	0.20	1.66	3.17
β-Ala	7.542	0.9993	1.00–100.00	0.20	1.06	5.00
Arg	8.887	0.9991	2.00–2000.00	0.10	2.89	4.19
Ala	9.375	0.9992	1.00–500.00	0.05	1.96	2.91
GABA	9.799	0.9998	1.00–200.00	0.05	0.99	3.43
Pro	10.285	0.9997	5.00–2500.00	0.20	3.88	8.07
Ammonium ion	11.895	0.9998	1.00–500.00	0.05	0.35	1.26
Ethanolamine	12.241	0.9998	0.10–100.00	0.05	0.84	2.27
Tyr	12.948	0.9991	1.00–100.00	0.20	1.27	3.94
Histamine	13.457	0.9985	0.50–100.00	0.04	1.79	2.28
Val	15.599	0.9998	1.00–500.00	0.10	2.04	4.59
Met	16.648	0.9994	1.00–50.00	0.10	2.32	3.15
Cys	18.268	0.9854	1.00–50.00	0.20	2.82	4.55
Ile	18.685	0.9994	1.00–100.00	0.10	2.21	5.37
Leu	19.281	0.9990	0.50–100.00	0.05	2.75	5.08
Trp	19.644	0.9993	1.00–100.00	0.10	2.03	2.99
Phe	20.407	0.9990	1.00–200.00	0.50	1.66	4.94
Orn	22.074	0.9998	1.00–100.00	0.20	1.72	3.97
Lys	22.824	0.9996	1.00–100.00	0.20	2.55	3.80
Agmatine	23.976	0.9982	0.50–50.00	0.10	0.62	2.06
Serotonin	24.508	0.9989	0.10–50.00	0.05	0.91	4.37
Tyramine	25.532	0.9981	0.10–50.00	0.05	0.80	2.65
Putrescine	26.412	0.9990	0.10–50.00	0.04	0.87	4.06
Cadaverine	26.738	0.9994	0.10–50.00	0.03	0.21	0.33
Phenylethylamine	26.859	0.9996	0.10–50.00	0.05	2.98	5.00
Spermidine	27.102	0.9993	0.10–50.00	0.03	0.96	4.95
Spermine	27.862	0.9991	0.10–50.00	0.08	1.02	4.38

[a] Limit of detection: signal/noise ratio = 3.
[b] RSD% of peak areas based on five runs of the same standard mixture within a single day.
[c] RSD% of peak areas based on five runs of the same standard mixture over independent days.

Table 3
Recoveries of the method for the determination of amino acids and biogenic amines in samples taken from five stages of fermentation. Recovery results were presented as mean value (with RSD% between brackets, $n = 3$).

Compound	Spiked level (mg/L)		Grape Must[a]			Early AF[b]		
			Initial concentration (mg/L)	Recovery (%)		Initial concentration (mg/L)	Recovery (%)	
	Low	High		Low	High		Low	High
Asp	5.56	22.24	85.74 (2.64)	111.05 (3.23)	87.84 (2.11)	27.12 (2.86)	92.63 (2.25)	89.29 (3.40)
Glu	29.80	119.20	47.14 (1.12)	115.73 (1.90)	116.84 (0.94)	40.70 (2.23)	88.35 (1.23)	102.70 (1.56)
Ser	5.32	21.26	45.29 (3.76)	84.29 (1.42)	101.42 (1.74)	39.66 (1.21)	103.37 (0.39)	104.73 (1.03)
Asn	10.65	42.60	19.13 (2.65)	103.16 (4.77)	107.99 (5.33)	12.23 (1.87)	97.08 (1.65)	110.38 (0.23)
Gln	10.94	43.76	92.49 (2.70)	88.36 (1.63)	103.14 (1.53)	82.86 (2.03)	108.78 (0.89)	111.49 (0.25)
His	10.64	42.56	75.80 (3.33)	86.64 (0.81)	109.84 (0.83)	47.82 (3.85)	82.15 (3.14)	106.58 (1.36)
Gly	10.15	40.58	45.40 (3.51)	109.51 (3.08)	82.75 (2.81)	3.46 (1.53)	81.99 (1.63)	104.74 (2.86)
Thr	5.05	20.20	76.63 (1.26)	86.58 (3.33)	96.31 (2.46)	23.40 (4.22)	91.04 (2.31)	100.58 (2.65)
β-Ala	5.12	20.48	21.68 (1.39)	83.67 (3.73)	101.36 (0.94)	21.14 (0.45)	102.01 (0.97)	115.59 (0.32)
Arg	25.25	101.00	473.03 (1.23)	95.85 (2.83)	97.05 (2.01)	294.87 (1.51)	97.11 (2.29)	80.80 (1.42)
Ala	23.80	95.20	162.19 (0.89)	89.99 (1.48)	103.42 (1.40)	35.66 (2.42)	85.35 (0.95)	86.98 (1.79)
GABA	12.88	51.50	49.44 (1.83)	96.05 (3.20)	100.03 (3.54)	41.82 (2.99)	105.46 (1.42)	105.26 (2.05)
Pro	552.02	1104.04	333.95 (4.10)	98.54 (2.73)	91.92 (2.38)	217.07 (1.53)	105.84 (2.72)	83.93 (2.87)
Ammonium ion	6.50	26.00	134.14 (0.80)	103.40 (1.62)	106.31 (1.50)	96.08 (1.15)	83.68 (0.95)	98.69 (0.98)
Ethanolamine	4.77	19.08	1.56 (8.72)	94.58 (2.46)	96.16 (2.46)	17.44 (0.82)	107.52 (2.37)	85.27 (1.52)
Tyr	5.13	20.50	10.20 (2.91)	89.13 (0.97)	89.64 (2.72)	6.17 (2.66)	98.21 (3.01)	89.98 (2.46)
Histamine	4.92	19.68	nd	93.05 (1.43)	85.77 (3.81)	nd	117.77 (5.65)	105.87 (3.25)
Val	5.03	20.12	60.37 (1.98)	89.33 (4.15)	106.58 (3.66)	15.44 (3.39)	113.22 (2.51)	93.04 (0.28)
Met	2.53	10.13	3.82 (2.70)	107.79 (3.95)	103.35 (1.32)	2.22 (3.03)	89.76 (1.87)	85.59 (2.41)
Cys	2.70	10.80	5.16 (4.68)	91.55 (1.89)	89.24 (3.60)	3.81 (4.67)	70.92 (3.65)	95.77 (1.81)
Ile	4.87	19.47	32.31 (2.96)	82.75 (3.25)	83.44 (3.94)	6.27 (2.92)	83.42 (0.96)	91.59 (0.36)
Leu	5.31	21.24	26.52 (2.58)	83.44 (2.74)	115.67 (1.69)	7.93 (1.98)	99.83 (3.21)	105.31 (1.32)
Trp	2.37	9.48	15.57 (4.22)	82.47 (2.07)	118.79 (1.00)	7.94 (0.85)	109.82 (1.27)	95.65 (1.58)
Phe	7.09	28.35	33.17 (2.22)	109.39 (1.19)	86.61 (1.29)	8.57 (0.94)	91.56 (0.16)	81.51 (0.42)
Orn	10.46	41.84	9.34 (1.09)	85.68 (0.89)	97.95 (1.17)	5.46 (2.73)	83.91 (3.59)	97.02 (1.75)
Lys	5.43	21.70	11.79 (0.88)	95.34 (2.02)	99.10 (3.69)	2.67 (4.31)	109.00 (4.07)	117.27 (4.90)
Agmatine	5.09	20.36	nd	100.73 (8.27)	112.92 (9.43)	nd	109.24 (8.38)	114.76 (5.83)
Serotonin	4.84	19.35	nd	103.93 (7.41)	84.45 (8.88)	nd	94.23 (8.73)	81.06 (5.20)
Tyramine	5.03	20.10	nd	98.32 (2.20)	100.45 (2.93)	1.23 (7.96)	86.96 (8.92)	82.47 (2.36)
Putrescine	5.27	21.06	1.69 (6.83)	96.48 (1.10)	88.55 (3.35)	4.29 (4.88)	93.90 (1.46)	80.17 (8.73)
Cadaverine	1.18	4.73	nd	99.43 (10.67)	86.76 (5.50)	nd	112.19 (8.03)	115.34 (3.79)
Phenylethylamine	5.24	20.95	1.56 (6.87)	103.23 (4.86)	101.66 (1.92)	0.20 (2.48)	86.54 (5.12)	80.78 (6.52)
Spermidine	5.51	22.02	nd	84.89 (1.07)	81.84 (3.21)	0.27 (8.12)	94.50 (8.54)	84.05 (8.17)
Spermine	4.82	19.26	nd	85.46 (8.89)	91.34 (8.23)	0.15 (10.67)	119.30 (8.81)	109.35 (10.96)

Compound	Mid AF[c]			End AF[d]			End MLF[e]		
	Initial concentration (mg/L)	Recovery (%)		Initial concentration (mg/L)	Recovery (%)		Initial concentration (mg/L)	Recovery (%)	
		Low	High		Low	High		Low	High
Asp	15.66 (1.99)	105.65 (3.60)	95.79 (0.08)	8.53 (3.40)	104.29 (4.76)	103.72 (11.33)	7.37 (4.34)	82.35 (3.63)	89.05 (3.90)
Glu	23.14 (4.59)	125.72 (3.77)	90.50 (3.67)	31.32 (4.46)	108.27 (2.88)	87.66 (2.00)	39.77 (3.77)	90.13 (2.14)	117.77 (2.24)
Ser	4.87 (4.36)	100.69 (4.35)	95.55 (3.68)	8.16 (1.90)	115.30 (3.45)	89.48 (2.06)	10.11 (1.79)	91.77 (0.65)	91.81 (0.69)
Asn	5.78 (1.21)	91.91 (3.47)	117.31 (1.89)	15.78 (2.81)	81.87 (2.83)	96.24 (6.68)	20.84 (0.89)	99.05 (0.73)	90.57 (0.31)
Gln	52.90 (3.34)	101.58 (1.53)	105.05 (1.44)	89.82 (1.62)	95.75 (2.21)	83.68 (12.57)	117.99 (1.00)	106.13 (0.11)	96.92 (1.15)
His	12.15 (2.16)	75.12 (2.37)	93.97 (0.37)	22.32 (1.89)	91.71 (2.92)	108.55 (2.48)	37.28 (0.96)	104.68 (2.32)	88.96 (0.18)
Gly	2.03 (4.87)	93.68 (3.79)	107.85 (4.09)	6.15 (3.45)	86.17 (1.40)	95.61 (2.36)	20.27 (1.69)	91.42 (1.15)	94.24 (0.03)
Thr	5.02 (3.64)	82.92 (1.64)	84.65 (1.28)	4.46 (1.31)	115.10 (3.97)	99.18 (3.17)	5.91 (2.85)	99.85 (0.16)	98.28 (1.70)
β-Ala	13.38 (4.73)	89.98 (0.07)	93.71 (0.15)	15.33 (1.53)	87.24 (2.38)	93.37 (4.20)	14.80 (1.57)	101.40 (1.88)	81.34 (1.82)
Arg	40.54 (1.42)	106.06 (0.23)	94.25 (0.13)	29.82 (0.81)	105.59 (3.23)	88.49 (4.04)	37.22 (1.26)	103.01 (1.89)	81.25 (1.75)
Ala	3.33 (4.69)	99.46 (0.90)	103.22 (2.75)	1.21 (1.48)	93.13 (3.29)	83.59 (4.43)	12.97 (3.39)	88.20 (0.35)	102.38 (1.30)
GABA	1.33 (1.32)	87.69 (0.49)	98.44 (0.42)	nd	97.73 (1.92)	103.08 (3.61)	21.69 (3.99)	95.44 (0.96)	86.32 (0.01)
Pro	839.39 (1.69)	88.70 (1.79)	84.90 (3.72)	1697.65 (1.40)	96.99 (4.10)	86.11 (2.42)	2061.71 (3.03)	105.10 (0.42)	88.88 (0.92)
Ammonium ion	11.95 (1.29)	93.14 (2.92)	91.06 (1.52)	1.69 (2.53)	107.48 (1.62)	94.11 (1.80)	2.18 (2.40)	109.13 (0.37)	96.31 (0.05)
Ethanolamine	16.44 (0.42)	114.16 (6.31)	118.15 (1.85)	18.83 (1.94)	80.83 (1.79)	88.34 (3.15)	24.25 (0.91)	88.86 (1.03)	91.65 (0.95)
Tyr	1.36 (5.02)	91.20	104.96	nd	103.41	86.25	3.09 (4.23)	99.97	95.02

(*continued on next page*)

Table 3 (*continued*)

Compound	Mid AF[c]			End AF[d]			End MLF[e]		
	Initial concentration (mg/L)	Recovery (%)		Initial concentration (mg/L)	Recovery (%)		Initial concentration (mg/L)	Recovery (%)	
		Low	High		Low	High		Low	High
		(2.22)	(3.48)		(2.10)	(1.92)		(0.94)	(1.08)
Histamine	nd	117.78	89.55	nd	81.46	84.74	1.63 (1.88)	83.62	81.82
		(4.08)	(6.50)		(4.87)	(3.95)		(0.59)	(1.44)
Val	7.71 (1.17)	86.46	97.79	1.14 (5.03)	91.47	92.57	3.27 (1.40)	99.67	89.79
		(2.24)	(1.52)		(1.99)	(1.66)		(0.42)	(0.73)
Met	nd	107.69	91.43	nd	89.84	86.57	5.34 (1.43)	88.61	97.01
		(4.69)	(2.86)		(1.52)	(4.33)		(0.62)	(0.18)
Cys	1.46 (2.60)	110.53	92.76	1.59 (3.46)	83.64	108.56	5.67 (3.16)	98.56	97.56
		(1.49)	(1.87)		(2.96)	(5.28)		(1.62)	(0.62)
Ile	1.39 (4.09)	105.05	105.29	3.26 (1.42)	89.89	104.32	3.58 (1.62)	95.24	92.84
		(1.37)	(0.85)		(1.38)	(2.50)		(1.45)	(0.08)
Leu	nd	105.62	85.99	2.75 (4.01)	93.62	99.99	2.67 (4.21)	97.99	81.41
		(0.63)	(0.55)		(1.90)	(0.95)		(1.69)	(1.35)
Trp	nd	88.32	88.89	1.66 (3.49)	105.87	101.87	6.17 (2.01)	85.53	114.45
		(3.81)	(2.85)		(4.03)	(3.14)		(2.03)	(1.89)
Phe	1.32 (5.50)	108.96	91.36	4.36 (1.19)	108.81	91.68	4.55 (1.23)	107.19	98.06
		(0.69)	(0.91)		(0.42)	(0.25)		(1.62)	(1.75)
Orn	2.59 (0.97)	91.85	104.65	2.35 (6.45)	90.15	98.83	13.55 (0.85)	103.78	94.47
		(1.88)	(2.18)		(0.96)	(0.10)		(0.98)	(2.11)
Lys	8.32 (2.97)	104.95	108.92	6.25 (2.96)	113.89	109.11	5.03 (5.88)	91.81	91.56
		(4.06)	(1.98)		(4.36)	(3.42)		(2.65)	(3.06)
Agmatine	nd	103.79	116.99	nd	87.86	105.11	22.83 (5.79)	109.35	119.14
		(10.47)	(4.48)		(5.80)	(4.34)		(3.55)	(6.90)
Serotonin	nd	85.11	93.15	nd	107.09	118.90	5.23 (6.91)	108.23	105.29
		(8.69)	(3.47)		(5.42)	(5.71)		(0.23)	(0.98)
Tyramine	0.75 (3.54)	80.95	85.37	0.17 (5.85)	111.31	118.07	0.69 (8.50)	117.91	107.53
		(6.95)	(2.52)		(3.16)	(9.50)		(1.36)	(0.28)
Putrescine	5.36 (0.51)	80.11	83.10	7.48 (0.13)	90.83	85.42	8.38 (0.81)	98.64	103.34
		(4.61)	(3.75)		(7.18)	(4.51)		(0.15)	(0.49)
Cadaverine	nd	100.82	91.48	nd	81.43	92.66	1.10 (9.49)	83.73	99.61
		(6.76)	(5.66)		(9.53)	(8.77)		(7.68)	(9.81)
Phenylethylamine	0.28 (2.01)	101.24	85.92	0.87 (5.80)	114.23	103.56	1.67 (9.51)	86.78	90.30
		(1.80)	(4.70)		(4.33)	(7.19)		(2.86)	(0.36)
Spermidine	1.93 (0.23)	106.52	82.32	1.15 (0.20)	82.20	84.04	3.67 (6.55)	99.13	103.99
		(4.16)	(2.89)		(2.75)	(8.00)		(1.78)	(3.76)
Spermine	0.18 (10.63)	111.45	115.26	0.26 (9.84)	84.71	80.68	nd	83.05	107.00
		(10.49)	(10.57)		(10.44)	(9.58)		(2.22)	(2.44)

nd, not detected.

[a] Grape must, 220.0 g/L of sugar.

[b] Wines taken from the second day of alcoholic fermentation (125.0 g/L of sugar).

[c] Wines taken from the middle stage of alcoholic fermentation (40.0 g/L of sugar).

[d] Wines at the end of alcoholic fermentation (<4.0 g/L of sugar).

[e] Wines at the end of malolactic fermentation (<4.0 g/L of sugar).

0 (must), 2 (early stage of alcoholic fermentation), 4 (middle stage of alcoholic fermentation) and 7 (end of alcoholic fermentation) of alcoholic fermentation, and the samples contained 220.0, 125.0, 40.0 and <4.0 g/L sugar, respectively. The samples that, at the end of malolactic fermentation, contained <4.0 g/L of sugar were also evaluated. In this condition, matrix-matched calibration curves prepared in 200.0, 100.0 and 0.0 g/L glucose solutions were used for the quantification of the first three fermentation stages, and matrix-free calibration curves were used for samples after the end of alcoholic and malolactic fermentation. All of the recovery results were shown in Table 3.

For most compounds and conditions, the recovery rates were in the range of 80% to 120%. Only Cys in the early stage of alcoholic fermentation sample, His and Glu in the middle stage of alcoholic fermentation sample showed recovery rates out of the range (70%, 75% and 125%, respectively). These results demonstrated that this method was accurate and reliable for the determination of amino acids and amines in wine samples during the fermentation process. The matrix-matched calibration curve was effective at correcting the incorrectly low quantification caused by the matrix influence in must and wine.

3.4. Application of the developed method to wine samples during fermentation

The developed method was used for the determination of amino acids and biogenic amines in commercial Cabernet Sauvignon wines collected at different stages of fermentation from a winery in Xinjiang, China. The wines were fermented in stainless steel tanks following a typical industrial red wine manufacturing process. At the end of alcoholic fermentation, three weeks of malolactic fermentation with grape skin maceration was carried out. We tracked the fermentation process of two different tanks (T1 150 hl and T2 200 hl) at five stages: musts at the beginning of alcoholic fermentation (must), wines on the second day of alcoholic fermentation (early AF), wines on the third day (T1 the third day and T2 the fourth day) of alcoholic fermentation (mid AF), wines at the end of alcoholic fermentation (end AF) and wines at the end of malolactic fermentation (end MLF). For the quantification of proline, ethanolamine and spermine, matrix-matched calibration curves prepared in 200.0, 100.0 and 50.0 g/L glucose solutions were used for the initial three stages, respectively. All analyses were performed in triplicate.

Table 4
Amino acids and biogenic amines concentrations (mean ± SD, $n = 3$) in wine samples taken from five stages of fermentation of two tanks (T1 and T2).

Compound	T1 (mg/L)					T2 (mg/L)				
	Must[a]	Early AF[b]	Mid AF[c]	End AF[d]	End MLF[e]	Must[a]	Early AF[b]	Mid AF[c]	End AF[d]	End MLF[e]
Asp	25.45 ± 0.61	10.84 ± 0.20	14.55 ± 0.38	10.28 ± 0.19	28.37 ± 0.42	26.43 ± 1.79	7.12 ± 0.39	12.13 ± 1.27	15.58 ± 0.47	34.30 ± 0.52
Glu	43.00 ± 0.18	27.01 ± 1.71	29.57 ± 0.24	12.55 ± 0.18	35.46 ± 0.65	38.91 ± 0.85	21.14 ± 0.92	28.40 ± 3.12	18.34 ± 0.44	35.21 ± 0.49
Ser	50.65 ± 1.26	2.93 ± 0.12	2.91 ± 0.07	3.77 ± 0.24	14.20 ± 0.29	52.68 ± 1.84	1.53 ± 0.07	1.27 ± 0.23	3.90 ± 0.12	15.02 ± 0.24
Asn	11.89 ± 0.63	8.26 ± 0.18	16.63 ± 0.13	13.38 ± 0.18	35.78 ± 0.79	9.73 ± 0.17	5.32 ± 0.18	10.00 ± 0.78	16.00 ± 0.23	29.07 ± 0.06
Gln	173.75 ± 3.82	72.52 ± 1.88	91.24 ± 1.97	104.48 ± 4.85	115.94 ± 1.71	143.83 ± 2.40	36.56 ± 1.09	74.56 ± 4.21	102.68 ± 0.05	103.55 ± 5.15
His	37.34 ± 2.13	9.06 ± 0.11	13.12 ± 0.25	31.70 ± 0.04	29.26 ± 0.44	25.51 ± 0.87	5.88 ± 0.04	7.75 ± 0.14	25.96 ± 0.44	25.17 ± 0.06
Gly	20.67 ± 1.16	3.26 ± 0.15	8.03 ± 0.24	13.46 ± 0.53	26.00 ± 0.28	3.62 ± 0.01	2.03 ± 0.07	6.15 ± 0.46	4.89 ± 0.30	23.51 ± 0.05
Thr	35.90 ± 1.92	5.04 ± 0.05	6.82 ± 0.10	4.25 ± 0.05	11.50 ± 0.13	30.31 ± 0.14	3.77 ± 0.02	5.07 ± 0.09	5.82 ± 0.17	12.45 ± 0.25
β-Ala	20.91 ± 0.74	17.20 ± 0.07	20.75 ± 0.29	24.05 ± 0.07	28.84 ± 0.50	21.72 ± 1.11	14.77 ± 0.22	14.04 ± 0.05	16.68 ± 0.52	24.85 ± 0.23
Arg	304.67 ± 10.24	47.14 ± 0.54	43.48 ± 0.31	27.74 ± 0.06	88.06 ± 1.54	314.87 ± 9.19	38.78 ± 0.96	26.59 ± 2.57	37.17 ± 0.76	29.00 ± 0.24
Ala	28.36 ± 1.15	4.63 ± 0.06	7.31 ± 0.07	6.15 ± 0.01	35.46 ± 0.89	34.46 ± 1.91	2.12 ± 0.07	3.56 ± 0.09	11.04 ± 0.32	36.35 ± 0.25
GABA	89.62 ± 3.38	7.10 ± 0.01	2.39 ± 0.04	nd	nd	96.23 ± 5.33	nd	nd	nd	nd
Pro	368.45 ± 25.18	784.41 ± 4.70	1424.74 ± 70.79	2158.24 ± 111.19	2354.75 ± 62.15	268.39 ± 2.69	724.45 ± 30.62	1731.01 ± 54.71	2281.57 ± 11.95	2494.38 ± 16.40
Ammonium ion	108.23 ± 2.81	1.12 ± 0.08	1.18 ± 0.04	4.94 ± 0.28	34.66 ± 0.87	128.90 ± 2.67	nd	1.30 ± 0.02	5.14 ± 0.28	63.74 ± 0.79
Tyr	10.47 ± 0.38	nd	nd	2.80 ± 0.10	14.35 ± 0.32	10.21 ± 0.28	nd	nd	3.64 ± 0.19	14.93 ± 0.18
Val	13.87 ± 0.78	nd	nd	nd	8.08 ± 0.24	15.22 ± 0.86	nd	nd	1.05 ± 0.05	8.84 ± 0.16
Met	1.99 ± 0.12	nd	nd	nd	5.43 ± 0.25	1.99 ± 0.10	nd	nd	nd	6.13 ± 0.03
Cys	1.51 ± 0.10	1.78 ± 0.05	1.98 ± 0.07	1.71 ± 0.09	3.70 ± 0.10	2.05 ± 0.03	1.23 ± 0.09	1.42 ± 0.05	1.98 ± 0.13	3.16 ± 0.07
Ile	9.98 ± 0.55	nd	4.85 ± 0.23	5.89 ± 0.40	9.03 ± 0.26	10.58 ± 0.49	nd	3.65 ± 0.22	3.60 ± 0.20	10.25 ± 0.16
Leu	14.02 ± 0.65	nd	1.37 ± 0.01	2.33 ± 0.13	17.98 ± 0.35	12.60 ± 0.59	nd	1.67 ± 0.04	3.01 ± 0.10	20.11 ± 1.01
Trp	7.26 ± 0.13	nd	nd	3.86 ± 0.14	12.96 ± 0.42	5.99 ± 0.15	nd	4.32 ± 0.31	3.47 ± 0.14	12.60 ± 0.34
Phe	8.46 ± 0.38	nd	nd	3.35 ± 0.06	13.03 ± 0.25	9.44 ± 0.26	nd	nd	3.85 ± 0.22	13.86 ± 0.56
Orn	9.09 ± 0.25	8.24 ± 0.04	7.93 ± 0.03	7.20 ± 0.03	33.85 ± 0.07	9.16 ± 0.42	5.31 ± 0.13	2.40 ± 0.08	7.00 ± 0.28	86.39 ± 1.65
Lyr	23.69 ± 0.63	8.43 ± 0.52	8.60 ± 0.47	5.05 ± 0.25	38.19 ± 1.61	25.10 ± 1.68	13.98 ± 0.85	3.67 ± 0.17	8.22 ± 0.31	37.97 ± 1.63
Ethanolamine	11.55 ± 0.85	16.38 ± 0.14	20.43 ± 0.17	26.35 ± 0.08	30.85 ± 0.54	11.24 ± 0.11	12.28 ± 0.11	20.04 ± 0.14	23.70 ± 0.74	28.98 ± 0.24
Histamine	nd	3.41 ± 0.05	6.57 ± 0.07	10.35 ± 0.56	23.13 ± 0.65	nd	2.72 ± 0.09	6.52 ± 0.19	10.06 ± 0.57	20.40 ± 0.74
Agmatine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Serotonin	nd	nd	nd	nd	3.27 ± 0.28	nd	nd	nd	nd	2.94 ± 0.03
Tyramine	2.84 ± 0.07	4.37 ± 0.08	4.36 ± 0.28	5.48 ± 0.29	6.49 ± 0.20	2.57 ± 0.12	3.88 ± 0.15	4.49 ± 0.10	5.48 ± 0.06	6.16 ± 0.61
Putrescine	4.77 ± 0.08	8.02 ± 0.24	9.49 ± 0.07	12.62 ± 0.49	12.11 ± 0.19	5.20 ± 0.25	5.85 ± 0.09	8.76 ± 0.20	10.84 ± 0.34	11.43 ± 0.13
Cadaverine	1.28 ± 0.04	1.58 ± 0.03	2.07 ± 0.11	3.46 ± 0.23	3.83 ± 0.10	1.83 ± 0.18	1.70 ± 0.12	2.34 ± 0.19	3.25 ± 0.04	3.40 ± 0.07
Phenylethylamine	1.47 ± 0.03	2.26 ± 0.07	2.89 ± 0.20	4.26 ± 0.30	4.77 ± 0.07	1.89 ± 0.07	2.31 ± 0.12	2.89 ± 0.15	3.12 ± 0.08	3.50 ± 0.10
Spermidine	4.52 ± 0.17	3.23 ± 0.02	4.73 ± 0.09	6.18 ± 0.35	9.43 ± 0.22	4.03 ± 0.12	2.61 ± 0.01	3.93 ± 0.19	4.07 ± 0.02	7.51 ± 0.62
Spermine	nd	nd	nd	nd	2.19 ± 0.02	nd	nd	nd	nd	1.97 ± 0.14

nd, not detected.
[a] Beginning of alcoholic fermentation.
[b] Early stage (second day) of alcoholic fermentation.
[c] Middle stage (third day of T1 and fourth day of T2) of alcoholic fermentation.
[d] End of alcoholic fermentation.
[e] End of malolactic fermentation.

The amino acids and biogenic amines concentrations in analysed samples were listed in Table 4. The analysis was repeatable with RSD < 7% for most of the analytes. Two fermentation processes showed similar features. In grape musts, the major amino acids were Arg, Pro, GABA, Gln and Ser, in addition to the ammonium ion. All other amino acids were present in amounts that did not exceed 50 mg/L. As they are the preferred nitrogen source for yeast (Bell & Henschke, 2005; Garde-Cerdán et al., 2011), most Arg and ammonium ion were consumed during the course of alcoholic fermentation. A large portion of GABA, Ser and Thr were also consumed by the yeast. However, Pro was released into the medium during the course of alcoholic fermentation, its concentration exceeded 2000 mg/L at the end of alcoholic fermentation. Other amino acids, such as Gln and His, were consumed at first but then were released into the medium during the second half of alcoholic fermentation. The production and release of some amino acids by yeast cells under certain conditions were thought to maintain normal oxidation–reduction balance (Mauricio, Valero, Millán, & Ortega, 2001; Valero et al., 2003). The most abundant five amino acids in wines after alcoholic fermentation were Pro, Gln, Arg, His and β-Ala. Other amino acids were present at less than 20 mg/L. When the malolactic fermentation finished, the concentrations of most amino acids increased, especially ammonium ion, Orn, Lyr and Ala. After the three weeks long malolactic fermentation with grape skin maceration, the yeast autolysis and extraction of amino acids from the grape skin might contribute to the increase in amino acids. Arg exhibited a considerable increase during malolactic fermentation in T1, meanwhile in T2 it decreased a bit. The differences of fermentation parameters such as temperature and oxygen dissolution in different fermentor capacity may contribute to the different trends.

Agmatine was not detected in any sample. Six amines (ethanolamine, tyramine, putrescine, cadaverine, phenylethylamine and spermidine) were found in the initial musts. These amines naturally presented in grapes and musts (Del Prete et al., 2009; Herbert et al., 2005; Lonvaud-Funel, 2001) and their levels may vary according to the grape variety, the degree of ripening, the soil type and composition (Peña-Gallego et al., 2012). The total biogenic amines present in musts accounted for about one-third of the content in the final wines after malolactic fermentation (27.5% in T1 and 31.0% in T2, shown in Supplementary Fig. 1). Most amines gradually accumulated during alcoholic fermentation. Only spermidine decreased slightly from must to the early stage of alcoholic fermentation in both tanks. There are studies demonstrated that wine yeast strains could produce histamine, ethanolamine, phenylethylamine, cadaverine and so on during the course of alcoholic fermentation (Caruso et al., 2002; Torrea & Ancín, 2002). The total biogenic amines produced during alcoholic fermentation accounted for 44.0% in T1 and 39.1% in T2 of the content in the final wines. Serotonin and spermine were only found in wines after malolactic fermentation. Histamine accumulated during malolactic fermentation accounted for more than 50% of its content in the final wines. Spermidine also accumulated a great proportion during malolactic fermentation. The other amines accumulated at relatively low levels. Malolactic fermentation influenced the level of biogenic amines according to many studies, especially tyramine and histamine (Hernández-Orte et al., 2006; Lonvaud-Funel, 2001; Manfroi et al., 2009; Soufleros, Bouloumpasi, Zotou, & Loukou, 2007). In our study, after the three weeks long malolactic fermentation with grape skin maceration, the biogenic amine concentrations of both tanks were found at relatively high levels.

Overall, amines that accumulated during the alcoholic fermentation process accounted for most of the amines content in the final wines, which indicated that in the samples analysed the presence of biogenic amines was linked to alcoholic fermentation more than must and malolactic fermentation.

4. Conclusion

The method developed was suitable for the simultaneous analysis of amino acids and biogenic amines in wines during the fermentation process. The chromatographic separation was achieved within 30 min, which was a substantial improvement of the analysis efficiency. The method was reproducible (RSD < 9%) and accurate (recovery rate in the range of 80–120%). Proline, ethanolamine and spermine derivatives showed remarkable matrix effect due to the presence of residual sugar. High concentrations of sugar affected the pH of the derivatisation reaction system. Matrix-matched calibration curve was efficient at correcting this effect. Using this method, we were able to monitor the changes in amino acids and biogenic amines during the fermentation process and provide reliable information for winemaking practice.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.04.064.

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