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Determination of Free Proline and Monosaccharides in Wine Samples by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)

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A sensitive and selective analytical method for the simultaneous separation and quantitative determination of proline and free monosaccharides in wine samples by high-performance anion-exchange chromatography coupled with pulsed amperometric detection is described. Under optimized experimental conditions, a complete separation was obtained in less than 30 min, using an isocratic elution with 10 mM NaOH and 1 mM $Ba(OAc)_2$. No postcolumn addition of strong bases to the eluent for enhancing detection sensitivity was needed. Upon 25-fold sample dilution and purification to avoid interference of tannins, pigments, and phenolic compounds, the fingerprinting of common monosaccharides (i.e., arabinose, glucose, fructose, galactose, and xylose) and proline in wines, musts, and vinegars can be easily accomplished. The method allows high recovery and satisfies the necessary requirements for accuracy, repeatability, and sensitivity. Values obtained for proline content ranged from 470 to 1190 mg/L in "Aglianico" red wines (mean value, 870 ± 192 mg/L, $n = 21$) and from 168 to 286 mg/L in white wines (mean value, 208 ± 32 mg/L, $n = 11$). Lower levels were found in musts of red and white grapes, 550 and 87 mg/L, respectively. The lowest content of proline, ca. 10 mg/L, was found both in white and red vinegars.

KEYWORDS: Wine; must; grapes; sugars; proline; chromatography; amperometric detection

1. INTRODUCTION

The introduction of novel analytical techniques, in conjunction with greater consumer demands and expectation for safer products, has given a tremendous impetus to food quality assurance (*1*). Authenticity of wine has been extensively investigated, because such a relatively low alcoholic beverage can be easily adulterated (*2*). Detailed and continuous controls are required to maintain the quality of wine during the entire wine-making process, as well as storage. The occurrence and composition of free sugars and amino acids, sugar ratios, and ratios between sugars and organic acids have proven to be very useful, providing information on the general quality, freshness, maturity, storability, and/or optimization of selected technological processes (*3*). Accordingly, accurate determinations of carbohydrates are gaining increasing significance, as they not only provide compositional information on samples but also assist in identifying adulteration, variety, origin, manufacturer, etc.

Nitrogen compounds, especially amino acids, are particularly important in enology with regard to their influence on the organoleptic profile and bouquet of wines (*4*). Free amino acids enhance taste and color of wine (*5*) and represent a group of substances for wine differentiation and classification (*6*). This was used to separate authentic Champagnes from sparkling wines, where the second fermentation to produce the overpressure of CO_2 is performed in the bottle, leading to an increase of amino acidic content (*7*). Proline, hydroxyproline, and ethanolamine showed clear differences between 34 French red wines (*8*). Amino acids in wines originate from various sources; indigenous compounds in grapes can be partially or totally metabolized by yeasts during the growth phase; some are excreted by living yeasts or released by proteolysis during the autolysis of dead yeasts, whereas others are produced by enzymatic degradation of grape proteins. It is also known that the amino acid content in wines is dependent upon the fertilization and climatic conditions as well as contact time of the grape skins during fermentation. The determination of amino acids is generally accomplished by high-performance liquid chromatography, which includes derivatization followed by UV−visible absorption or fluorescence emission detection of the corresponding derivatives (*9*−*14*). Pre- and postcolumn derivatization both suffer from several drawbacks, such as relatively long analysis times, low stability of amino acid derivatives, formation of multiple adducts, and reagent interference (*15*). Hence, the development of a robust, highly sensitive, and direct method to determine the most significant amino acids,

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and especially to assay proline in wine samples, would be very useful and desirable.

High-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) has become increasingly popular in recent years (*16*−*18*), and some examples of sugar determinations in wine samples exist (*19*, *20*). Indeed, the very good match between liquid chromatography and pulsed amperometry has allowed the determination of carbohydrates in a variety of complex matrixes, such as foods, beverages, vegetal tissues, and even in the area of clinical diagnostics, being also very promising for the determination of sugars and amino acids occurring free in wine samples. Current analytical methods for wine sugars lack the capacity to simultaneously separate and quantify free monosaccharides, proline, and other amino acids (*21*). Here, we describe a direct, accurate, and simultaneous quantitative determination of glucose, fructose, galactose, xylose, and arabinose, along with threonine, proline, and hydroxyproline. The method combines a rapid isocratic separation, achieving high levels of sensitivity, accuracy, and reproducibility. Using a simple cleanup procedure, our method will be demonstrated to be effective and reliable, allowing evaluation of the sugar profile and proline content of red and white wines, as well as grape juices and vinegars. Special attention was given to "Aglianico del Vulture" red wines, which are among the most produced and popular red wines in our region (Basilicata, South Italy).

2. MATERIALS AND METHODS

2.1. Chemicals. Chemical standards of the various carbohydrates (D-glucose, 99.5%, D-galactose, 99%, D-arabinose, 99%, D-xylose, 99%, D-fructose, 98%) and amino acids (l-proline, 99%, l-hydroxyproline, 99%, l-threonine, 99%) were purchased from Sigma-Aldrich (Steinheim, Germany); carbonate-free sodium hydroxide (50% w/w), barium acetate (99%) and sodium azide were also obtained from Sigma-Aldrich (Steinheim, Germany). The internal standard, 2-deoxy-D-glucose (99%), and methanol of HPLC grade were purchased from Fluka (Buchs, Switzerland). Stock solutions were prepared with pure water supplied by Milli-Q RG unit from Millipore (Bedford, MA). All the reagents used in this study were of the highest purity available. Sodium hydroxide solutions used as eluents were prepared by dilution of a carbonate-free 50% (w/w) NaOH solution in water, previously filtered with a 0.45-μm membrane and degassed with nitrogen. The exact concentration of hydroxide ions in the mobile phases was determined by titration against a standard solution of hydrochloric acid.

2.2. Samples and Sample Preparation. Musts and commercial wines from different brands were kindly supplied by local producers: Azienda Agricola Paternoster, Aglianico del Vulture (Rionero in Vulture, PZ, Italy), and Azienda Agricola Mustilli di L. Mustilli, (Sant'Agata dei Gooti, BN, Italy). Additional samples of wine, as well as bottles of vinegar (white, red and balsamic), were bought in local markets. All the sample bottles were refrigerated until used, protected from sunlight, and opened only at the moment of analysis. The sample cleanup was that described by Bernal et al. (*20*) with slight modification. Briefly, classical Sep-Pak C_{18} SPE cartridges (Waters SpA, Milan, Italy) were preconditioned by sequential treatment with methanol (2 × 5 mL) and water (2 × 5 mL). Samples were properly diluted with 20 μM internal standard, and an aliquot of each sample (5 mL) was passed through the preconditioned SPE cartridges. The final eluate was filtered using 0.22-μm membrane filters (Whatman plc, Kent, UK) and injected. It was possible to use one cartridge for at least three analyses with identical performance.

2.3. Apparatus and Method. Chromatographic separations were performed on a Dionex Instrument (Sunnyvale, CA), equipped with a metal-free isocratic pump (Model IP 20), a Rheodyne injection valve (model RH9125, Cotati, CA) with a 10-μL loop, and a pulsed amperometric detector (model ED40). The flow-through detection cell is made from a 1.0-mm diameter gold working electrode and a pH-Ag|AgCl combination reference electrode (set to Ag mode); the titanium body of the cell served as the counter electrode. All the separations were realized using a Dionex column CarboPac PA1 (25 × 4 mm i.d.), coupled with a guard column (5 × 4 mm i.d.). During all the experiments, the flow rates were 1.0 mL/min, and the column temperature was kept at 24±1 °C, using a homemade water jacket coupled with a circulating water bath model WK4DS from Colora (Colora, Messtechnik GmbH, Germany). The analytical and guard columns were regenerated at the beginning of each working day by washing with 200 mM NaOH for ca. 30 min. The compounds of interest were separated using an isocratic elution with 10 mM NaOH and 1 mM barium acetate (*22*). Barium acetate was dissolved in deionized water, and after sparging with N_2 for 15 min, the required amount of NaOH (50% w/w solution) was added. Sparging was continued for 2 min to mix the mobile phase. The plastic reservoir bottles (DX 500 2 L bottles, Dionex) were closed and pressurized with pure nitrogen to 0.8 MPa. A six-potential waveform, as proposed by Clarke et al. (*23*), was employed for the detection of sugars and amino acids (see **Table 1**). Data acquisition and processing was performed using the PeakNet Chromatography Workstation software, version 5.1.

Table 1. Potential-Time Settings for Sugar and Amino Acid Detection According to Clark et al. (*23*)

	potential vs Ag/AgCl (V)	time (s)	current integration
E_1	−0.20	0.00	
	−0.20	0.04	
E_2	−0.05	0.05	
	−0.05	0.11	begin
E_3	0.28	0.12	
	0.28	0.41	
E_4	−0.05	0.42	
	−0.05	0.56	end
E_5	−2.00	0.57	
	−2.00	0.58	
E_6	0.60	0.59	
	−0.20	0.60	

2.4. Standard and Working Solutions. Stock solutions of 10 mM of all the analytes were prepared in water, stabilized with 0.1% sodium azide to prevent microbial growth and stored at 4 °C. Working standard solutions were prepared on the day of use by dilution with water. Aliquots of these solutions were treated as the samples. The sugar and amino acid contents of samples were obtained by interpolation on the standard curves.

3. RESULTS AND DISCUSSION

3.1. The Mobile Phase Choice. The mobile phase composition in HPAEC significantly affects the selectivity of separation, as well as the sensitivity of detection (*24*). Sodium hydroxide solutions are usually employed, and the hydroxide concentration to be used depends on the class of compounds under investigation. The analysis of mono and oligosaccharides is carried out with strongly alkaline eluents ([OH−] ≥ 0.1 M) as these compounds strongly interact with the anion-exchangers of the stationary phase (*18*). In an attempt to separate monosaccharides and amino acids in the same chromatographic run, the use of alkaline eluents at low hydroxide concentrations (10−12 mM) was successfully evaluated. As previously reported (*22*), the addition to dilute alkaline eluents of minute amounts of barium acetate (1−2 mM) has been demonstrated to be very effective in minimizing the effects of carbonate ion, also positively affecting the separation of carbohydrates. Barium ions, in alkaline eluents, are able to form carbonate salts, which precipitate in the mobile phase container (pK_s $BaCO_3$ = 8.30), simply providing a chemical means for the removal of carbonate ions. Thus, in a series of experiments, the applicability of an

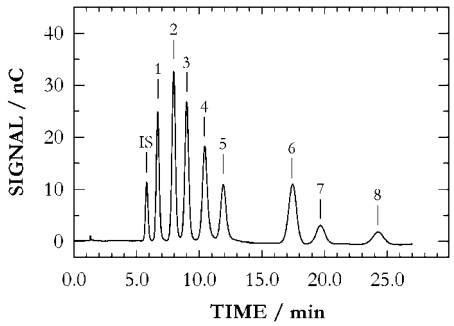

Figure 1. Separation in HPAEC with pulsed amperometric detection of a mixed standard solution containing arabinose (1), galactose (2), glucose (3), xylose (4), fructose (5), threonine (6), proline (7), and hydroxyproline (8) at the concentration of 40 μM each; the internal standard (IS) is 2-deoxyglucose. Eluent, 10 mM NaOH + 1 mM $Ba(OAc)_2$ at a flow rate of 1.0 mL/min. Column, Dionex CarboPac PA1 plus guard column. The potential waveform detailed in **Table 1** was employed at the gold working electrode.

eluent solution composed of 10 mM NaOH and 1 mM barium acetate for the separation of sugars and some amino acids identified in wines was investigated. A representative example of separation is reported in **Figure 1**. Details concerning the potential waveform employed in pulsed amperometric detection at the gold working electrode are described in the following section.

3.2. Electrochemical Detection in Pulsed Amperometry. A three-potential waveform, generally applied to amperometrically detect sugar compounds (*17*, *25*), is not suitable to determine low concentrations of amino acids, as anodic detection of amine-containing compounds requires the electrocatalytic activity of oxide species on the gold electrode surface. At the detection potential, surface stabilized oxidation with anodic oxygen transfer occurs concomitantly with the formation of surface oxides. Hence, oxidation of preadsorbed analyte is the main signal contribution, even though the gold oxide formation strongly affects the background current. To minimize such an effect, several potential waveforms have been proposed to detect amino and thiocompounds (*26*−*28*). Although Johnson and coworkers in 1989 (*29*) introduced the first integrated pulsed amperometric detection (IPAD), only recently, Clarke et al. (*23*) demonstrated the superiority of an integrated amperometric six-step potential waveform over conventional IPAD to detect amino acids and amino sugars in HPAEC. Such a potential waveform was found to successfully minimize baseline shifts during gradient elution, to improve linearity and long-term reproducibility, and to increase the signal-to-noise ratio. Moreover, the electrode cleaning at negative potential allows a clean and active electrode surface to be maintained without causing electrode recession and loss of signal (*30*). As illustrated in **Figure 1**, the pulsed amperometric detection of monosaccharides and selected amino acids at the gold working electrode in the same chromatographic run was accomplished by employing a six-step potential waveform, which is detailed in **Table 1**.

3.3. Solid-Phase Isolation and Recovery. To verify the efficacy of isolation and recovery of sugars and proline in solid-phase extraction (SPE), a standard mixture at the concentration of 100 μM each was passed through a C_{18} cartridge, previously activated by methanol and water. Upon filtration, the eluate was injected and analyzed by HPAEC-PAD. A separate portion of the same mixture, without passing through the cartridge, was injected, and recoveries of proline, glucose, and fructose were evaluated by comparison of peak areas. No differences were observed, confirming that the compounds of interest are not retained by the C_{18} cartridges. Moreover, two aliquots of the same wine, at different dilution (1:1 and 1:25), were injected in triplicate after SPE treatment. A slightly reduced signal (ca. <3%) was obtained using the 25-fold sample dilution before SPE, which in turn extends the lifetime of cartridges. This means that each SPE cartridge could be used for at least three samples, also avoiding the analytical column overloading by pigments, tannins, and phenolic compounds occurring in wines. The recovery of proline, glucose, and arabinose in red and white wines was within the range of 95 ± 3−98 ± 6%. On this basis, all wine samples were diluted 25-fold before passing through the cartridge.

Table 2. Reproducibility of Retention Times for Intra- and Interday Analysis of a Red Wine Sample by HPAEC-PAD[a]

	intraday ($n = 5$)		interday (7 days, $n = 21$)	
compound	t_r (min)	%RSD	t_r (min)	%RSD
arabinose	6.7	0.9	6.9	1.6
galactose	8.0	1.1	8.4	1.9
glucose	9.0	0.8	9.4	2.4
xylose	10.5	1.1	10.9	2.9
fructose	11.9	1.2	12.3	2.7
threonine	19.5	1.4	20.1	3.5
proline	20.3	1.7	20.9	3.7
hydroxyproline	25.6	2.3	26.5	4.7

[a] Separation conditions as those in **Figure 1**.

Table 3. Calibration Parameters (signal = $m\,c + b$) of Carbohydrates and Amino Acids Evaluated by HPAEC-PAD[a]

compound	$m \pm$ SD	$b \pm$ SD	r	linear range μM; (n)[b]
arabinose	0.080 ± 0.001	-0.3 ± 0.1	0.99968	5 / 200 (9)
galactose	0.121 ± 0.002	-0.4 ± 0.2	0.99926	5 / 200 (9)
glucose	0.109 ± 0.002	-0.33 ± 0.15	0.99949	5 / 200 (8)
xylose	0.094 ± 0.001	-0.32 ± 0.13	0.99943	5 / 200 (9)
fructose	0.060 ± 0.001	-0.21 ± 0.13	0.99962	5 / 200 (8)
threonine	0.087 ± 0.001	-0.14 ± 0.01	0.99958	5 / 150 (8)
proline	0.029 ± 0.001	-0.04 ± 0.03	0.99947	5 / 150 (8)
hydroxyproline	0.027 ± 0.004	-0.05 ± 0.03	0.99924	5 / 150 (7)

[a] Concentration values expressed as μmol/L. 2-deoxy-D-glucose was used as an internal standard. Chromatographic conditions as those in **Figure 1**. [b] Number of standard solutions used for calibration.

3.4. Reproducibility of Retention and Calibration Data. **Table 2** summarizes the precision of retention times observed upon injecting the same sample of red wine and using the optimized experimental conditions. Excellent precision was obtained by repeating the analysis of the same red wine five times. As can be seen, relative standard deviations (RSDs) of retention times were lower than 2.3% ($n = 5$) for the same day, while these values increase up to 4.7% when the same experiment was repeated in seven different days ($n = 21$). The RSDs of peak areas were better than 2.6%. Calibration curves were constructed by plotting the peak area ratios of each compound to IS as a function of solute concentration. The calibration data of compounds considered of value in wine samples are summarized in **Table 3**. The standard deviation (SD) of slope and intercept was estimated at the 95% confidence level. Such an accurate and highly sensitive chromatographic method, combined with a simple sample cleanup, shows potential for quantitative determination of carbohydrates and amino acids in the micromolar range.

The optimized experimental conditions were thus applied to the analysis and profiling of sugars and amino acids present in

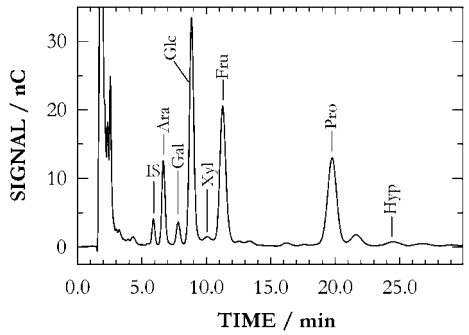

Figure 2. HPAEC-PAD separation of a red wine sample, Portali 1996. Sample diluted 25-fold before injection. Other experimental conditions as those for **Figure 1**.

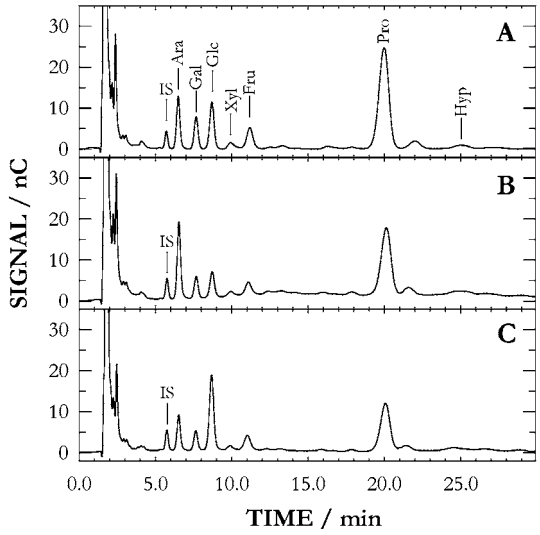

Figure 3. HPAEC-PAD separations of red wines (1:25) from the cultivar (Aglianico): vintages 1997 (A), 1998 (B), and 1999 (C). Other conditions as those for **Figure 1**.

wine, must, and vinegar samples. The good separation capability of the method is demonstrated in **Figure 2**, where a representative chromatogram of a sample of red wine from vintage 1996 is reported. The following compounds were identified: arabinose, galactose, glucose, xylose, fructose, proline, and hydroxyproline. As can be seen, the chromatographic separation of wine samples is interference-free, with few peaks clearly recognizable; 2-deoxy-D-glucose was selected as an internal standard.

3.5. Determination of Proline and Free Sugars in Wines. Thirty-two wine samples, both red (*21*) and white (*11*) were processed in order to verify a possible correlation of the free sugar and amino acid content versus vintage. First of all, a set of red wines was selected, coming from the same producer (Paternoster), who used the same type of grapes (Aglianico) and the same technological treatment for the vinification throughout the chosen period of time. **Figure 3** shows the chromatographic separations of three following vintages, namely 1997, 1998, and 1999, see plots (A), (B), and (C), respectively. In addition to the internal standard, the chromatograms exhibit seven well-separated peaks, whose retention times match those of arabinose (Ara), galactose (Gal), glucose (Glc), xylose (Xyl), fructose (Fru), proline (Pro), and hydroxyproline (Hyp). Other injections were made with samples that were spiked with small amounts of xylose and hydroxyproline for accurate identification. Moreover, to establish the possible coelution of different analytes, the following compounds were injected: mannose, sucrose, arginine, lysine, glycine, and alanine. Whereas arginine elutes in the column void volume, lysine, glycine, and alanine elute in the time window shown, but their content is not elevated in wines (*31*), and detection sensitivity is relatively low. However, a partial peak overlapping between xylose/mannose and glucose/sucrose was noted. As far as the first couple, xylose in wine is much more abundant than mannose, which cannot be detected in diluted samples. Also, for the second couple, there are no problems, as sucrose is normally absent in wine samples, being quickly hydrolyzed during the fermentation process.

Table 4. Mean Content (mg/L), Minimum, Maximum and Standard Deviation of Free Monosaccharides and Some Amino Acids Evaluated by HPAEC-PAD in Red ($n = 21$) and White ($n = 11$) Wines

	red wine[a]			white wine[b]		
compound	min	max	mean ± SD	min	max	mean ± SD
arabinose	35	120	72 ± 16	5	40	18 ± 18
galactose	22	85	48 ± 18	10	65	26 ± 14
glucose	5	220	62 ± 40	20	162	124 ± 60
xylose	5	45	14 ± 9	5	27	10 ± 9
fructose	23	315	122 ± 75	25	178	148 ± 65
threonine	3	30	16 ± 6	10	32	26 ± 6
proline	470	1190	870 ± 192	168	286	208 ± 32
hydroxyproline	100	240	170 ± 45	40	112	96 ± 55

[a] All red wines were obtained from local producers of Aglianico red grapes, kindly supplied by Azienda Agricola Paternoster and Azienda Agricola Mustilli.[b] White wines were always DOC (controlled and guaranteed denomination of origin) wines collected from producers in the South of Italy.

The mean values of free monosaccharides and amino acids found in red and white wines are compiled in **Table 4**. Large variability was observed in the sugar content of wines investigated in this study. Whereas xylose was the least abundant monosaccharide, arabinose and/or glucose occurred in considerable amounts. As expected, proline was the most abundant among the amino acids analyzed. Such an amino acid is present in the mature fruit of *Vitis vinifera*, and accumulates in the berries during maturation (*32*). Its absolute level is not affected by alcoholic fermentation, and for these reasons it has been suggested as a molecular marker of grape variety (*33*). Within all red wines investigated, the level of proline was found to range from 470 to 1190 mg/L, with higher concentration in the vintage of 1997. It is interesting to note that the content of proline in Merlot and Cabernet Italian red wines was well within such a range, 952 mg/L and 672 mg/L, respectively (*6*). Even though no significant differences in the sugar composition were obtained by analyzing wines of the same vintage and grape variety, distinctive sugar profiles (fingerprinting) were observed. This feature was confirmed when red wines of a second local producer of Aglianico wine (i.e., Mustilli) were analyzed (not shown). Most likely, such a large variability may be due to several factors, such as ripeness grade, local environmental conditions, manufacturing procedures, and storage.

Eleven samples corresponding to young white wines elaborated in the south of Italy were also assayed. These wines are made from the free running grape juice, without the grape mash, having no contact with the grape skin. In **Figure 4,** a representative chromatogram of a sample of white wine, Pipoli 2000, is presented. All the analytes were identified by retention times and standard addition. Here, the sugar content variability could be attributed to the different ripeness of grapes. As expected, the mean content of proline in white wines is comparably lower than that of red wines, 208 ± 32 and 870 ± 192 mg/L, respectively. These findings are in agreement with those of other authors who have observed that the amount of

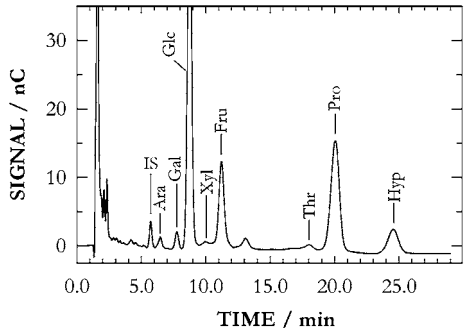

Figure 4. HPAEC-PAD separation of a white wine sample, Pipoli 2000. Sample diluted 25-fold before injection. Other conditions as those for **Figure 1**.

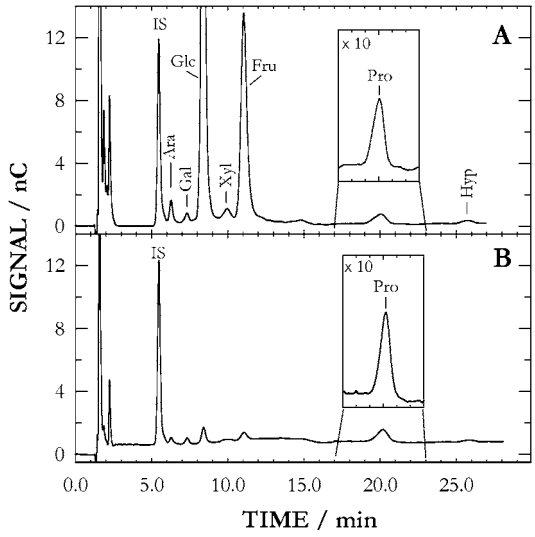

Figure 5. HPAEC-PAD of two vinegars, (A) red and (B) white. Each sample was diluted 20-fold before injection. Other conditions as those for **Figure 1**.

each amino acid varies widely, according to variety (*34*,*35*), yeast and bacteria strain, region, technological treatment, and age (*36*). Red wines are richer in proline than white ones, as this amino acid is present in the pulp and peel of grapes, thus it is more efficiently extracted during vinification of red grapes. Analogous considerations are valid for arabinose, which is much more abundant in red wines. High contents of glucose and fructose, coming from the pulp of berries, were observed in white wines, confirming that the level of these compounds is influenced by their origin in grapes and winemaking techniques.

3.6 Determination of Proline and Free Sugars in Musts and Vinegars. Preliminary separations by HPAEC-PAD were also accomplished on some Italian musts and vinegars. The mean composition of monosaccharides and amino acids was evaluated as the average of three measurements performed on different amounts of the same sample. Both white and red musts, upon 50-fold dilution with water, exhibit almost equal amounts of glucose and fructose (not shown). Obviously, their levels are relatively higher than that of wine samples, where these compounds are consumed during vinification. As already demonstrated in the case of wines, proline is comparably less abundant in white than in red grape juices, 87 and 550 mg/L, respectively.

Figure 5 compares the chromatograms of two commercially available white and red vinegars. The mean contents of sugars and amino acids found in vinegars are listed in **Table 5**. A different sugar profile was obtained, the red sample being

Table 5. Proline and Sugar Contents (mg/L±SD) of Three Selected Grape Vinegars as Determined by HPAEC-PAD

compound	red	white	balsamic
arabinose	7.7 ± 0.5	1.9 ± 0.2	39 ± 2
galactose	4.3 ± 0.2	3.1 ± 0.2	39 ± 2
glucose	319 ± 10	9.5 ± 0.3	(12.2 ± 0.5)10^3
xylose	7.7 ± 0.5	1.9 ± 0.2	18 ± 1
fructose	183 ± 10	5.8 ± 0.5	(9.1 ± 0.5)10^3
threonine	NQ[a]	NQ[a]	NQ[a]
proline	9.3 ± 0.5	11.4 ± 0.5	142 ± 5
hydroxyproline	2.3 ± 0.2	NQ[a]	NQ[a]

[a] Not quantified.

particularly rich in glucose and fructose, while there were no significant differences in terms of proline content between white and red vinegars. Finally, **Table 5** reports an additional example of this method applied to a *balsamic* vinegar, which contains huge amounts of glucose and fructose, in addition to a relatively high level of proline, ca. 142 mg/L. Such data confirm that pulsed amperometric detection in conjunction with HPAEC is a valid and effective methodology for the simultaneous determination of monosaccharides and selected amino acids in grape juices, musts, and wines during storage and aging.

Although special attention has been devoted to the determination of proline, which is the most important amino acid in wine, we are continuously developing the HPAEC-PAD methodology. The issues that are currently being addressed include the evaluation of the full amino acidic profile for wine characterization. For this purpose, a new sample preparation method will be attempted (*37*−*39*). It includes the use of a strong cation exchanger, prior to HPAEC-PAD determination, which would be very useful to separate amino acids from carbohydrates and other neutral components occurring in wine samples.

4. CONCLUSIONS

An improved knowledge of the carbohydrate and amino acid compositions of wines may be obtained by HPAEC-PAD, which is convenient, sensitive, and very reliable. The developed method seems to be very suitable to assess the influence of technological steps on the content of free sugars in grape juices, wines, and vinegars, along with their evolution during fermentation, storage, and aging of wine. Proline determination represents a versatile alternative to established chromatographic methods, in which pre- or postcolumn derivatization is usually employed. It has been confirmed that red wine is an abundant source of proline.

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