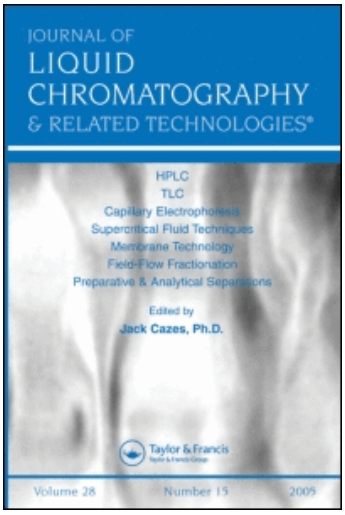


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# HPLC Determination of Free Amino Acids Profile of Dão Red Wine: Effect of *Dekkera bruxellensis* Contamination

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**Abstract:** A reversed-phase HPLC/UV-Vis methodology for determination of twenty free amino acids in Dão red wine is described. The sample preparation was simple, involving only a pre-column derivatization with dabsyl chloride. The HPLC/UV-Vis procedure is sensitive, reproducible, and accurate. The detection limit values for free amino acids were low, between 0.01 and 0.07 μg/mL, and the method was precise. As a general rule, the recovery values were high.

This methodology was applied to the evaluation of the effect of the spoilage wine yeast *Dekkera bruxellensis* on the Dão red wine free amino acids profile. This yeast seems to influence the free amino acids composition of the wine, including aspartic

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and glutamic acids, asparagine, glutamine, and isoleucine as the main affected compounds.

**Keywords:** Dão red wine, *Dekkera bruxellensis*, Free amino acids



## INTRODUCTION

Free amino acids from grape must are used as nutrients for the growth of microorganisms such as yeast and bacteria, being consumed as source of nitrogen during alcoholic and secondary fermentations, thus contributing to the organoleptic properties of wine. In fact, they correspond to an important fraction of the total nitrogen in must and wine (from 30 to 40%).[1–3] The free amino acids profile depends on several factors, such as grape variety, geographic origin, field treatment and viticulture practices, production year, and wine-making technologies.[1–4] Amino acids in wine arise from several sources. Besides those present in the grapes, which can be partially or totally metabolized by yeast during the growth phase, some are produced by enzymatic degradation of grape proteins. Others are excreted by yeasts at the end of the fermentation or released by proteolysis during the autolysis of dead yeasts.[2,3]

Yeasts have an important role in wine production, and several species present the capacity to adulterate the wine. The presence of *Dekkera bruxellensis* in wines is one of the main economical problems associated to the wine industry. This yeast produces several types of compounds, such as ethylphenols, leading to significant organoleptic alterations, characterized as smoke, horse, or stable odour.[5–7] Once malolactic and alcoholic fermentation is completed, these spoilage yeasts grow easily on traces of residual sugars. This problem usually occurs during aging prior to bottling, but careful hygiene and adequate sulphuring of wines and containers can prevent its occurrence.[5,6]

The Dão region (north central Portugal) has important viticulture traditions, with secular reputation. The vineyards from Dão that give origin to wines with "*Denomination d'Origine Controllée"* (DOC) correspond to 20,000 ha and are implanted in granite land, between 400 and 500 m altitude, with unique climatic conditions. The grape varieties destined to the elaboration of DOC "Dão" wines are divided in two groups: the authorized varieties, that are disappearing and practically only exist in the oldest vineyards, and the recommended ones, that are the most important of the region. Touriga Nacional is a much esteemed red variety from the latter, because of its capacity to produce high quality wines.

In spite of the socio-economical importance they represent for this region, as far as we know, only DOC "Dão" wine obtained from Touriga Nacional variety was studied by our group, in what concerns the non-coloured phenolics, anthocyanins, organic acids, and volatile phenols composition and the influence of *D. bruxellensis* yeast in these chemical constituents.[8,9]

Several analytical methods have been reported for the determination of free amino acids in wine, involving gas chromatography (GC) or high performance liquid chromatography (HPLC), with pre- or post-column derivatization with various reagents. HPLC separations can be achieved either with ion-exchange or reversed-phase columns and the amino acid derivatives are mainly detected by fluorescence or UV-Vis absorbance.[1–4,10–12] Among these methods, pre-column derivatization with dabsyl chloride has been successfully used in the analysis of the amino acids composition of complex matrices.[13]

In the present paper, an analytical method was developed and validated for the determination of free amino acids in Dão red wine obtained from Touriga Nacional grapes, by HPLC/UV-Vis after pre-column derivatization with dabsyl chloride. The method was then applied to samples inoculated with *D. bruxellensis*, in order to evaluate the effect of this yeast on the free amino acids profile of the wine.



## EXPERIMENTAL

### Standards and Reagents

All L-amino acid standards, dabsyl chloride reagent, sodium hydrogen carbonate, sodium dihydrogenphosphate, dimethylformamide, and triethylamine were from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile, ethanol, and phosphoric acid were obtained from Merck (Darmstadt, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other reagents were of analytical grade from several suppliers.

### Microorganisms

Various *D. bruxellensis* strains were isolated from red wine from Dão in Sabouraud medium with chloramphenicol (Pronadisa, Spain). Yeasts were identified as previously reported by their biochemical characteristics, according to the PROLEWINE system.[8]

### Wine Samples

Monovarietal red wine obtained from Touriga Nacional grapes growing in the Dão region was treated as described before.[8] Briefly, 5 L of wine was taken from a stainless steel tank, sterilised by filtration, and distributed into 12 glass flasks. Samples W and WR were used as witness. Each one of the D samples was inoculated with a different *D. bruxellensis* strain. Sample S was

inoculated with *Saccharomyces cerevisiae* and was used as a control. Several colonies of each strain were suspended in sterile water up to a concentration of $8 \times 10^6$ cells/mL and aliquots of 2 mL were inoculated in each glass flask. All samples were kept in an oven at 18°C for three weeks, with the exception of sample WR, which was stored in a refrigerator (3°C) and was used to evaluate the effect of the temperature (Figure 1). About $1.5 \times 10^6$ CFU/mL were counted at the end of the incubation of the inoculated samples. The samples were then kept frozen until analysis.

## Sample Preparation/Derivatization Procedure

When dealing with liquid matrices, sample preparation was very simple, involving only the derivatization procedure. The dabsylation was achieved as previously reported by Krause et al.[13] although some modifications were made to the original method.

Aliquots of 20 μL of standard solutions (ca. 0.2 mg/mL of each amino acid in HCl 0.1 M) or wine samples were diluted with 180 μL of the reaction buffer (sodium hydrogen carbonate 0.15 M, pH 8.6 with NaOH). After thorough mixing on a vortex-mixer, 200 μL of dabsyl chloride reagent 12.4 mM (in acetone) were added and the vials were mixed again. The resulting solutions were incubated at 70°C in a water bath, during 15 min. The reaction was stopped by placing the vials in an ice bath for 5 min. 400 μL of the dilution buffer [mixture of 50 mL acetonitrile +25 mL ethanol + 25 mL sodium dihydrogenphosphate 9 mM, dimethylformamide

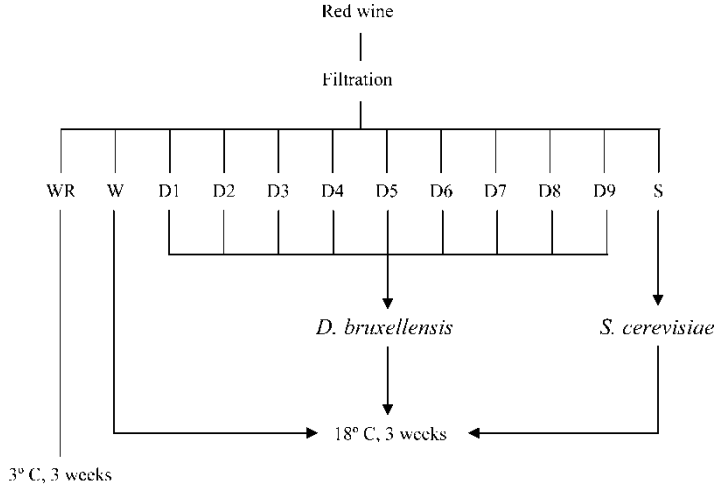


***Figure 1.*** Characterization of the analysed wine samples. W: witness sample; WR: refrigerated witness sample; D1–D9: samples inoculated with different *D. bruxellensis* strains; S: sample inoculated with *S. cerevisiae.*

4% and triethylamine 0.15% (pH 6.55 with phosphoric acid)] were added, followed by thorough mixing and centrifugation (5 min, 5,000 rpm). The clear supernatants were directly set for injection or stored at $-20^{\circ}$C.

### HPLC/UV-Vis Analysis

Dabsyl derivatives of free amino acids were separated on a Gilson HPLC unit, using a reversed-phase Spherisorb ODS2 column (25.0 × 0.46 cm; 5 μm particle size), according to Krause et al.[13] but with some modifications in the original gradient. The solvent system consisted of sodium dihydrogenphosphate 9 mM, dimethylformamide 4%, and triethylamine 0.15% (pH 6.55 with phosphoric acid) (A) and 80% acetonitrile (B). Elution was performed at a flow rate of 1 mL/min, starting with 20% B and installing a gradient to obtain 20% B at 7 min, 35% B at 35 min, 50% B at 45 min, and 100% B at 66 min. Detection was achieved with a UV-Vis detector set at 436 nm. Free amino acids quantification was accomplished by the absorbance recorded in the chromatograms relative to external standards. 20 μL of the derivatized standard solutions or samples were injected.



### Statistical Analysis

The evaluation of statistical significance was determined by ANOVA, followed by the Newman-Keuls test. The level of significance was set at $p < 0.05$.

## RESULTS AND DISCUSSION

### Analytical Curves and Detection Limits

Under the described assay conditions, a linear relationship between the concentration of amino acids and the absorbance at 436 nm was obtained in the tested range (Table 1). The correlation coefficient for the standard curves invariably exceeded 0.987, for all the compounds.

The detection limits were calculated as the concentration corresponding to three times the standard deviation of the background noise, and the values obtained were low, once they ranged from 0.01 to 0.07 μg/mL (Table 1).

### Validation of the Method

The proposed reversed-phase HPLC/UV-Vis methodology was specially developed for the determination of free amino acids profile in Dão red wine

***Table 1.*** Equations for regression lines and correlation coefficients, concentration range of linearity and detection limits for amino acids.

| Amino acid | Equation | Linearity (μg/mL) | Detection limit (μg/mL) |
|---|---|---|---|
| Aspartic acid | $y = 6.43 \times 10^5$ x; r = 0.99658 | 26.7–213 | 0.03 |
| Glutamic acid | $y = 3.85 \times 10^5$ x; r = 0.99984 | 24.3–194 | 0.05 |
| Asparagine | $y = 7.65 \times 10^5$ x; r = 0.98741 | 24.0–192 | 0.03 |
| Glutamine | $y = 8.31 \times 10^5$ x; r = 0.99766 | 22.8–182 | 0.02 |
| Serine | $y = 1.05 \times 10^6$ x; r = 0.99812 | 25.6–204 | 0.02 |
| Threonine | $y = 5.92 \times 10^5$ x; r = 0.99966 | 25.4–203 | 0.03 |
| Glycine | $y = 3.15 \times 10^6$ x; r = 0.99678 | 24.4–195 | 0.01 |
| Alanine | $y = 2.23 \times 10^6$ x; r = 0.99796 | 24.9–199 | 0.01 |
| Valine | $y = 1.46 \times 10^6$ x; r = 0.99854 | 24.6–197 | 0.01 |
| Proline | $y = 2.04 \times 10^6$ x; r = 0.99922 | 25.6–205 | 0.01 |
| Arginine | $y = 6.56 \times 10^5$ x; r = 0.99763 | 25.0–200 | 0.03 |
| Isoleucine | $y = 1.46 \times 10^6$ x; r = 0.99988 | 24.9–199 | 0.01 |
| Leucine | $y = 1.58 \times 10^6$ x; r = 0.99849 | 22.6–181 | 0.01 |
| Tryptophan | $y = 1.10 \times 10^6$ x; r = 0.99918 | 23.0–184 | 0.02 |
| Phenylalanine | $y = 1.19 \times 10^6$ x; r = 0.99844 | 23.5–188 | 0.02 |
| Cysteine | $y = 2.74 \times 10^5$ x; r = 0.99717 | 24.1–193 | 0.07 |
| Ornithine | $y = 2.22 \times 10^6$ x; r = 0.99756 | 24.2–193 | 0.01 |
| Lysine | $y = 2.18 \times 10^6$ x; r = 0.99872 | 22.6–181 | 0.01 |
| Histidine | $y = 1.21 \times 10^6$ x; r = 0.99776 | 30.1–244 | 0.02 |
| Tyrosine | $y = 2.35 \times 10^6$ x; r = 0.99871 | 21.6–172 | 0.01 |

y-peak area at 436 nm; x-μg of amino acid; r-correlation coefficient.



and to evaluate the effect of the spoilage wine yeast *Dekkera bruxellensis* on its free amino acid composition.

In order to validate the procedure and to assess its applicability to the routine analysis of these food products, several Dão red wine samples were analysed by the proposed technique, which allowed the separation, identification, and quantification of twenty free amino acids: aspartic acid, glutamic acid, asparagine, glutamine, serine, threonine, glycine, alanine, valine, proline, arginine, isoleucine, leucine, tryptophan, phenylalanine, cysteine, ornithine, lysine, histidine, and tyrosine (Table 2 and Figure 2). As far as we know, this is the first time that these compounds are reported in wine from Touriga Nacional grapes and from the Dão region.

The precision of the analytical method was evaluated by measuring the peak chromatographic area of free amino acids six times on the same sample (W). The standard deviations and the coefficients of variation (%) of these compounds are presented in Table 3. The analytical method was considered precise, once the coefficients of variation were low (between 0.53 e 5.54%; n = 6).

***Table 2.*** Free amino acids composition of Dão wine samples (mg/L) (quantification by external standard technique)

| | Samples | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WR | | W | | D1 | | D2 | | D3 | | D4 | | D5 | | D6 | | D7 | | D8 | | D9 | | S | |
| Amino acid | Mean[a] | SD | Mean[a] | SD | Mean[a] | SD | Mean[a] | SD | Mean[a] | SD | Mean[a] | SD | Mean[a] | SD | Mean[a] | SD | Meana | SD | Mean[a] | SD | Mean[a] | SD | Mean[a] | SD |
| Aspartic acid | 28.6 | 0.17 | 21.5 | 0.72 | 7.3 | 0.77 | 22.0 | 1.46 | 3.6 | 0.26 | 18.8 | 0.30 | 2.7 | 0.07 | 6.2 | 0.07 | 31.4 | 1.19 | 3.9 | 0.08 | 1.8 | 0.06 | 7.0 | 0.25 |
| Glutamic acid | 5.8 | 0.45 | 4.9 | 0.17 | 28.8 | 2.16 | 8.0 | 0.82 | 35.8 | 3.39 | 36.2 | 0.82 | 24.1 | 0.15 | 31.4 | 1.50 | 44.3 | 2.19 | 55.2 | 4.63 | 41.0 | 3.18 | 17.2 | 0.63 |
| Asparagine | 52.3 | 3.68 | 6.3 | 0.35 | 16.7 | 1.75 | 15.5 | 0.69 | 9.6 | 0.55 | 18.0 | 1.61 | 16.7 | 0.73 | 6.3 | 0.07 | 5.5 | 0.15 | 19.4 | 0.07 | 6.6 | 0.49 | 5.7 | 0.08 |
| Glutamine | 4.5 | 0.08 | 2.1 | 0.11 | 13.5 | 0.90 | 5.8 | 0.47 | 4.3 | 0.37 | 4.9 | 0.18 | 4.0 | 0.22 | 4.3 | 0.21 | 10.7 | 0.07 | 8.8 | 0.03 | 3.8 | 0.26 | 6.9 | 0.50 |
| Serine | 7.2 | 0.32 | 5.3 | 0.14 | 5.8 | 0.45 | 5.0 | 0.36 | 3.9 | 0.03 | 6.9 | 0.25 | 5.2 | 0.13 | 3.7 | 0.08 | 2.7 | 0.07 | 5.2 | 0.25 | 9.2 | 0.18 | 2.3 | 0.07 |
| Threonine | 5.6 | 0.08 | 3.9 | 0.13 | 4.2 | 0.06 | 4.2 | 0.16 | 2.8 | 0.10 | 6.3 | 0.48 | 3.9 | 0.27 | 2.9 | 0.11 | 3.5 | 0.06 | 3.7 | 0.19 | 4.6 | 0.42 | 3.4 | 0.04 |
| Glycine | 12.1 | 0.53 | 9.7 | 0.29 | 12.6 | 0.71 | 11.4 | 0.20 | 9.3 | 0.25 | 13.8 | 1.35 | 9.6 | 0.60 | 6.7 | 0.23 | 11.7 | 0.20 | 12.1 | 0.85 | 25.5 | 0.13 | 12.4 | 0.93 |
| Alanine | 32.7 | 0.99 | 24.2 | 0.60 | 23.0 | 0.66 | 25.4 | 0.87 | 19.4 | 1.02 | 28.3 | 1.25 | 20.5 | 1.20 | 13.6 | 0.19 | 32.9 | 1.14 | 24.1 | 0.67 | 27.9 | 0.65 | 25.4 | 0.81 |
| Valine | 4.6 | 0.11 | 3.6 | 0.15 | 5.8 | 0.10 | 3.8 | 0.07 | 4.0 | 0.04 | 4.9 | 0.27 | 3.5 | 0.25 | 2.3 | 0.03 | 4.8 | 0.25 | 4.5 | 0.29 | 6.2 | 0.21 | 5.2 | 0.14 |
| Proline | 524.8 | 9.20 | 518.2 | 2.75 | 620.1 | 35.72 | 527.7 | 23.18 | 572.5 | 5.31 | 559.3 | 10.56 | 531.5 | 22.92 | 386.4 | 20.79 | 515.6 | 6.18 | 524.4 | 6.11 | 482.7 | 5.12 | 490.8 | 14.38 |
| Arginine | 13.8 | 1.31 | 9.9 | 0.22 | 13.4 | 0.59 | 5.7 | 0.09 | 6.4 | 0.38 | 10.2 | 0.61 | 9.2 | 0.54 | 7.8 | 0.39 | 8.0 | 0.10 | 16.0 | 0.26 | 17.4 | 1.10 | 20.0 | 2.11 |
| Isoleucine | 2.9 | 0.09 | 1.4 | 0.07 | 3.4 | 0.06 | 4.5 | 0.21 | 4.6 | 0.07 | 6.1 | 0.07 | 2.9 | 0.14 | 1.5 | 0.02 | 2.0 | 0.03 | 3.6 | 0.01 | 4.4 | 0.13 | 3.9 | 0.21 |
| Leucine | 3.4 | 0.15 | 2.1 | 0.07 | 1.7 | 0.05 | 2.8 | 0.09 | 3.3 | 0.11 | 3.2 | 0.15 | 2.5 | 0.19 | 2.3 | 0.03 | 2.0 | 0.19 | 3.9 | 0.17 | 4.8 | 0.09 | 4.5 | 0.38 |
| Tryptophan | 6.0 | 0.12 | 4.3 | 0.17 | 3.5 | 0.17 | 4.5 | 0.32 | 3.7 | 0.09 | 6.8 | 0.08 | 6.4 | 0.01 | 3.9 | 0.32 | 5.5 | 0.13 | 1.4 | 0.12 | 10.2 | 0.22 | 12.3 | 1.22 |
| Phenylalanine | 10.4 | 0.36 | 7.7 | 0.16 | 6.4 | 0.59 | 5.5 | 0.43 | 5.5 | 0.09 | 11.0 | 0.22 | 4.6 | 0.21 | 5.7 | 0.48 | 9.5 | 0.50 | 8.9 | 0.69 | 6.5 | 0.25 | 6.4 | 0.51 |
| Cysteine | 30.9 | 1.60 | 26.0 | 0.83 | 20.6 | 1.37 | 27.1 | 1.66 | 22.4 | 1.96 | 24.2 | 0.77 | 5.6 | 0.09 | 21.0 | 1.46 | 26.4 | 0.30 | 22.8 | 1.67 | 9.5 | 0.41 | 24.4 | 0.38 |
| Ornithine | 2.2 | 0.13 | 1.4 | 0.05 | 3.5 | 0.08 | 2.3 | 0.09 | 2.6 | 0.24 | 1.7 | 0.04 | 1.5 | 0.14 | 1.8 | 0.09 | 2.0 | 0.03 | 2.0 | 0.14 | 2.6 | 0.02 | 2.0 | 0.01 |
| Lysine | 13.6 | 0.42 | 9.5 | 0.18 | 10.6 | 0.29 | 11.2 | 0.92 | 9.2 | 0.71 | 13.7 | 0.86 | 7.9 | 0.73 | 5.7 | 0.19 | 14.1 | 0.51 | 10.4 | 0.74 | 15.2 | 0.44 | 11.5 | 0.15 |
| Histidine | 5.7 | 0.06 | 3.1 | 0.07 | 2.7 | 0.20 | 4.5 | 0.22 | 2.4 | 0.14 | 4.1 | 0.07 | 3.2 | 0.33 | 2.5 | 0.02 | 5.8 | 0.20 | 3.3 | 0.16 | 5.3 | 0.22 | 4.6 | 0.18 |
| Tyrosine | 3.0 | 0.23 | 3.2 | 0.12 | 4.6 | 0.23 | 6.8 | 0.54 | 3.9 | 0.30 | 4.1 | 0.11 | 5.7 | 0.06 | 3.6 | 0.04 | 4.2 | 0.29 | 4.9 | 0.09 | 4.0 | 0.24 | 3.9 | 0.25 |
| Total | 770.2 | | 668.3 | | 808.3 | | 703.6 | | 729.3 | | 782.3 | | 670.9 | | 519.7 | | 742.5 | | 738.6 | | 689.1 | | 669.8 | |

[a]Mean value found for three assays; SD-standard deviation.

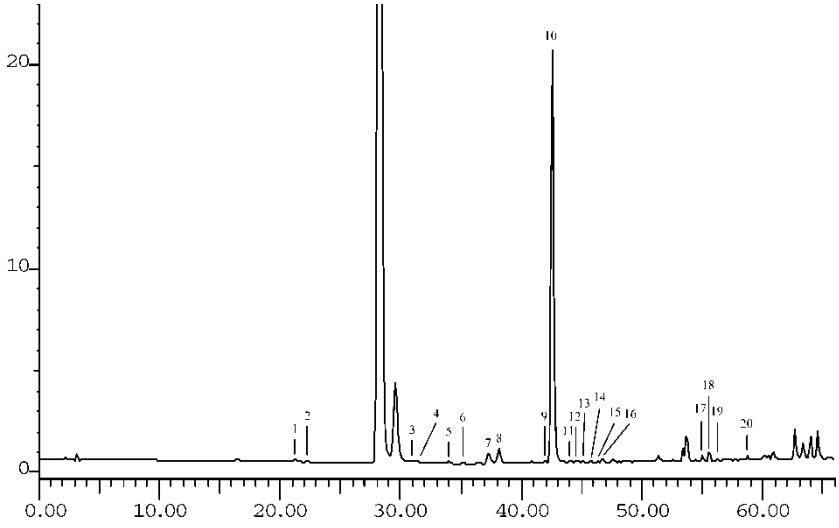


***Figure 2.*** HPLC/UV-Vis chromatogram of the free amino acids in a Dão red wine sample. Detection at 436 nm. (1) aspartic acid; (2) glutamic acid; (3) asparagine; (4) glutamine; (5) serine; (6) threonine; (7) glycine; (8) alanine; (9) valine; (10) proline; (11) arginine; (12) isoleucine; (13) leucine; (14) tryptophan; (15) phenylalanine; (16) cysteine; (17) ornithine; (18) lysine; (19) histidine; (20) tyrosine.

In order to study the recovery of the procedure, the same Dão red wine sample (W) was added to three known quantities of each standard (Table 4). The sample was analysed in triplicate, before and after the additions. Generally, the recovery values were high (between 75 and 117%), which demonstrates the effectiveness and the accuracy of the proposed method.

## Effect of *Dekkera bruxellensis* Contamination on Dão Red Wine Free Amino Acid Profile

All the analysed samples exhibited a common qualitative profile, composed by the twenty mentioned free amino acids, with proline as the major compound, varying from 68 to 79% of total compounds (Figure 3). This is not surprising, considering that this amino acid is the main compound in grape juice.[14] In addition, it has also been found to be the major free amino acid in other Portuguese elementary red wines.[4]

Considering the effect of the refrigeration, the results showed no influence of this factor on the free amino acids total amount, but a higher content of asparagine in the sample stored at low temperature (WR) was clearly observed (Table 2 and Figure 3).

In the samples inoculated with *D. bruxellensis*, the total free amino acids amount presented a mean value of ca. 709 mg/L. When comparing with the

***Table 3.*** Evaluation of the analytical method precision (n = 6) (quantification by external standard technique)

| Amino acid | SD (mg/L) | CV (%) |
|---|---|---|
| Aspartic acid | 0.72 | 3.36 |
| Glutamic acid | 0.17 | 3.40 |
| Asparagine | 0.35 | 5.54 |
| Glutamine | 0.11 | 4.93 |
| Serine | 0.14 | 2.65 |
| Threonine | 0.13 | 3.41 |
| Glycine | 0.29 | 2.99 |
| Alanine | 0.60 | 2.48 |
| Valine | 0.15 | 4.25 |
| Proline | 2.75 | 0.53 |
| Arginine | 0.22 | 2.21 |
| Isoleucine | 0.07 | 5.42 |
| Leucine | 0.07 | 3.57 |
| Tryptophan | 0.17 | 3.84 |
| Phenylalanine | 0.16 | 2.12 |
| Cysteine | 0.83 | 3.19 |
| Ornithine | 0.05 | 3.83 |
| Lysine | 0.18 | 1.86 |
| Histidine | 0.07 | 2.42 |
| Tyrosine | 0.12 | 3.69 |

SD-standard deviation; CV-coefficient of variation.



results of the witness sample (W), it could be noticed that some strains, such as D6, lead to a reduction of the free amino acids content, while others, like D1, induced an increase in its amount (Table 2). In a general way, the inoculation with *D. bruxellensis* seems to affect, mainly, the contents of aspartic and glutamic acids, asparagine, glutamine, and isoleucine, causing an increase in the levels of the last four compounds (Table 2 and Figure 3), which may be explained by a release of these amino acids. The lower content noticed for aspartic acid (Table 2 and Figure 3) is possibly due to its assimilation by the yeast. In addition, some differences in the free amino acids profile resultant from the inoculation with the distinct *D. bruxellensis* strains were observed. For example, concerning tryptophan, which represented ca. 0.6% of total amino acids in the witness sample (W), D8 strain led to a decrease of its relative amount (to ca. 0.2%), but D9 caused an increase to ca. 1.5%. The same happened with arginine, representing ca. 1.5% of total compounds in the W sample, which decreased in the presence of D2 and D3 strains (to ca. 0.9%), while inoculation with D8 and D9 strains resulted in a higher relative amount (ca. 2.3%).

In general, the inoculation with either *D. bruxellensis* or *S. cerevisiae* resulted in a similar effect. However, when comparing the results between

***Table 4.*** Recoveries of free amino acids from a spiked Dão wine sample (W) (quantification by external standard technique).

| Amino acid | Present (mg/L) | Added (mg/L) | Found[a] (mg/L) | SD (mg/L) | CV (%) | Recovery (%) |
|---|---|---|---|---|---|---|
| Aspartic acid | 21.5 | 5.1 | 24.8 | 0.28 | 1.13 | 93.2 |
| | | 10.1 | 25.8 | 0.54 | 2.11 | 81.6 |
| | | 20.3 | 36.1 | 3.12 | 8.65 | 86.4 |
| Glutamic acid | 4.9 | 4.6 | 8.0 | 0.46 | 5.80 | 84.2 |
| | | 9.3 | 13.6 | 0.69 | 5.06 | 95.8 |
| | | 18.5 | 21.9 | 0.12 | 0.57 | 93.6 |
| Asparagine | 6.3 | 4.6 | 8.5 | 0.64 | 7.51 | 78.0 |
| | | 9.1 | 17.6 | 0.29 | 1.66 | 114.3 |
| | | 18.2 | 24.6 | 1.21 | 4.93 | 100.4 |
| Glutamine | 2.1 | 4.3 | 6.8 | 0.59 | 8.80 | 106.3 |
| | | 8.7 | 11.1 | 0.73 | 6.55 | 102.8 |
| | | 17.3 | 19.3 | 0.32 | 1.66 | 99.5 |
| Serine | 5.3 | 4.9 | 8.4 | 0.10 | 1.14 | 82.4 |
| | | 9.7 | 11.2 | 0.73 | 6.51 | 74.7 |
| | | 19.5 | 19.3 | 1.39 | 7.18 | 77.8 |
| Threonine | 3.9 | 4.8 | 7.6 | 0.06 | 0.74 | 87.4 |
| | | 9.7 | 11.9 | 0.86 | 7.21 | 87.5 |
| | | 19.3 | 22.4 | 0.45 | 2.03 | 96.6 |
| Glycine | 9.7 | 4.6 | 15.5 | 0.85 | 5.44 | 108.4 |
| | | 9.3 | 19.0 | 0.28 | 1.47 | 100.0 |
| | | 18.6 | 27.9 | 2.84 | 10.19 | 98.6 |
| Alanine | 24.2 | 4.7 | 29.2 | 0.24 | 0.83 | 101.0 |
| | | 9.5 | 30.1 | 2.43 | 8.08 | 89.3 |
| | | 18.9 | 34.7 | 0.74 | 2.12 | 80.5 |
| Valine | 3.6 | 4.7 | 7.7 | 0.14 | 1.84 | 92.8 |
| | | 9.4 | 11.8 | 0.40 | 3.38 | 90.8 |
| | | 18.7 | 22.7 | 0.33 | 1.43 | 101.8 |
| Proline | 518.2 | 4.9 | 514.6 | 17.12 | 3.33 | 98.4 |
| | | 9.7 | 537.2 | 15.74 | 2.93 | 101.8 |
| | | 19.5 | 529.4 | 39.93 | 7.54 | 98.5 |
| Arginine | 9.9 | 4.8 | 11.4 | 0.46 | 4.04 | 77.6 |
| | | 9.5 | 18.1 | 0.22 | 1.21 | 93.3 |
| | | 19.0 | 26.6 | 2.04 | 7.67 | 92.0 |
| Isoleucine | 1.4 | 4.7 | 5.2 | 0.27 | 5.22 | 85.2 |
| | | 9.5 | 10.5 | 0.45 | 4.29 | 96.3 |
| | | 18.9 | 20.5 | 1.69 | 8.26 | 101.0 |
| Leucine | 2.1 | 4.3 | 6.3 | 0.47 | 7.39 | 98.4 |
| | | 8.6 | 10.7 | 0.40 | 3.73 | 100.0 |
| | | 17.2 | 17.7 | 0.73 | 4.12 | 91.7 |

(*continued*)

***Table 4.*** Continued

| Amino acid | Present (mg/L) | Added (mg/L) | Found[a] (mg/L) | SD (mg/L) | CV (%) | Recovery (%) |
|---|---|---|---|---|---|---|
| Tryptophan | 4.3 | 4.4 | 9.9 | 0.22 | 2.21 | 113.8 |
| | | 8.7 | 14.0 | 0.91 | 6.52 | 107.7 |
| | | 17.5 | 21.9 | 1.20 | 5.49 | 100.5 |
| Phenylalanine | 7.7 | 4.5 | 11.6 | 0.35 | 3.00 | 95.1 |
| | | 8.9 | 16.6 | 0.68 | 4.11 | 100.0 |
| | | 17.9 | 24.9 | 2.22 | 8.91 | 97.3 |
| Cysteine | 26.0 | 4.6 | 26.4 | 2.20 | 8.32 | 86.8 |
| | | 9.2 | 37.1 | 2.94 | 7.91 | 105.4 |
| | | 18.4 | 46.0 | 0.86 | 1.86 | 103.6 |
| Ornithine | 1.4 | 4.6 | 7.0 | 0.09 | 1.25 | 116.7 |
| | | 9.2 | 8.1 | 0.20 | 2.50 | 76.4 |
| | | 18.4 | 15.2 | 0.34 | 2.24 | 76.8 |
| Lysine | 9.5 | 4.3 | 10.7 | 0.30 | 2.78 | 77.5 |
| | | 8.6 | 15.0 | 0.80 | 5.34 | 82.9 |
| | | 17.3 | 24.6 | 0.61 | 2.44 | 91.8 |
| Histidine | 3.1 | 5.8 | 7.4 | 0.62 | 8.37 | 83.1 |
| | | 11.6 | 13.2 | 0.68 | 5.14 | 89.8 |
| | | 23.3 | 21.2 | 0.43 | 2.01 | 80.3 |
| Tyrosine | 3.2 | 4.1 | 6.4 | 0.19 | 2.92 | 87.7 |
| | | 8.2 | 9.5 | 0.56 | 5.92 | 83.3 |
| | | 16.4 | 16.7 | 0.64 | 3.84 | 85.2 |

[a]Mean value found for three assays for each studied concentration; SD-standard deviation;

CV: coefficient of variation.



them, it can be noticed that the last yeast significantly increased the amounts of arginine and tryptophan (Table 2). Additionally, *S. cerevisae* seems to have no effect in asparagine, which was one of the main free amino acids affected by *D. bruxellensis* (Table 2 and Figure 3).

## CONCLUSIONS

In conclusion, the proposed HPLC/UV-Vis procedure for free amino acids profile determination is simple, sensitive, precise, reproducible, and accurate. This method has the main advantage of being very rapid in terms of sample preparation, which is very easy, involving only a pre-column derivatization with dabsyl chloride. The procedure reported herein is suitable for routine analysis in wine quality control determinations, namely for the evaluation

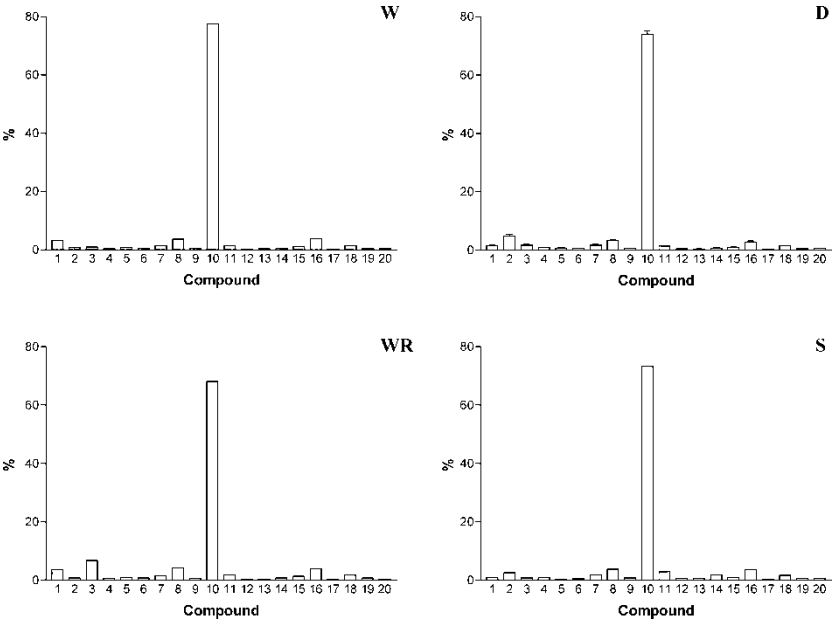


***Figure 3.*** Free amino acids profiles of witness sample (W), samples inoculated with *D. bruxellensis* strains (D), refrigerated witness sample (WR) and sample inoculated with *S. cerevisiae* (S). Values represent mean and standard error bars are on the top of each column. (1) aspartic acid; (2) glutamic acid; (3) asparagine; (4) glutamine; (5) serine; (6) threonine; (7) glycine; (8) alanine; (9) valine; (10) proline; (11) arginine; (12) isoleucine; (13) leucine; (14) tryptophan; (15) phenylalanine; (16) cysteine; (17) ornithine; (18) lysine; (19) histidine; (20) tyrosine.



of the spoilage wine yeast *Dekkera bruxellensis* influence upon the contents of free amino acids in Dão red wine. *D. bruxellensis* seems to affect the free amino acids profile of Dão red wine, being aspartic and glutamic acids, asparagine, glutamine, and isoleucine contents the most influenced.

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