# AMINOACIDS PROFILES IN WINES WITH LOW CONTENT OF SULPHUR DIOXIDE FROM PANCIU WINE REGION

## DISTRIBUȚIA UNOR AMINOACIZI IN VINURILE CU UN CONTINUT SCĂZUT DE DIOXID DE SULF DIN PODGORIA PANCIU

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***Abstract.*** *In wine industry, amino-acids play an important role in one of the most important elements of wine-making as fermentation and, as a result, are involved in specificity of wine variety. In the present study, specific wine verities from Panciu wine region were included, namely: Fetească regală, Fetească regală Frizzante, Cabernet Sauvignon and Cabernet Sauvignon rose. Every type of wines was processed by classic wine making methodologies but also with a different a modified scheme in order to compensate the role of low sulphur dioxide wine variants. Levels of amino-acids were determined by GC-MS, using a specific derivatisation method and ratio of intensities of peaks, permitted the quantification of 16 principal aminoacids. Some of aminoacids as alanine, glycine, valine, leucine, threonine, lysine and serine were highly affected in terms of decrease of concentration for low sufur dioxide wines. Proline which was resulted from the fermentation process had values higher than the average content of other aminoacids, from 1515.93 mg/L to 157 mg/L. Comparing the varieties with low and standard value of sulphur-dioxide, the levels of amino acids were differently affected. As a conclusion, the analysis of samples showed that the levels of amino-acids did not affect the overall quality of some the wines, the decreased levels can be associated with the transformation on other metabolites as biogenic amines.*
**Key words:** low sulphur dioxide wines, GC-MS, amino-acids

***Rezumat.*** *În industria vinului, aminoacizii joacă un rol important într-unul dintre cele mai importante elemente ale vinificației, în etapa de fermentație, astfel sunt implicați în definirea specificității unui vin de soi. În studiul de față, au fost incluse o serie de soiuri de vin din podgoria Panciu, precum: Fetească regală, Fetească regală Frizzante, Cabernet Sauvignon și Cabernet Sauvignon rose. Fiecare variantă de vin a fost vinificată prin metoda clasică, dar și prin ajustări, pentru a compensa cantitatea redusă de dioxid de sulf a produsului finit. Nivelurile de aminoacizi au fost determinate prin GC-MS, folosind o metodă specifică de derivatizare, iar raportul intensităților ionilor specifici, a permis identificarea a 16 aminoacizi. Unii dintre aminoacizii precum alanina, glicina, valina, leucina, treonina, lizina și serina au*

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*fost puternic afectați în ceea ce privește scăderea concentrației pentru vinurile cu conținut redus de dioxid de sulf. Prolina rezultată din procesul de fermentare a avut valori mai mari decât conținutul mediu al altor aminoacizi, de la 1515,93 mg/L până la 157 mg/L. Comparând vinurile obținute cu conținut redus dioxidului de sulf cu cele obținute prin metoda clasică s-a constatat că aminoacizii au fost influențați de tehnologia aplicată. Ca o concluzie, analiza probelor a arătat că aminoacizii nu au afectat calitatea generală a vinurilor, cantitățile scăzute putând fi asociate cu transformarea în alți metaboliți precum amine biogene.*

**Cuvinte cheie:** vinuri cu conținut scăzut de dioxid de sulf**,** GC-MS, amino-acizi

## INTRODUCTION

Amino acids are compounds with mixed function that contain two types of functional groups with a specific side chain. Amino acids are considered to be of natural occurance if they are aliphatic. In practice there are 20 amino acids which are key components of proteins namely alanine, valine, leucine, isoleucine, proline, tryptofan, metionine, serine, arginine, hystidine, threonine, serine, tyrosine, aspragine, glutamine, cisteine, aspartic acid glutamic acid, arginine, lisine, phenylalanine, valine (Reddy *et al.,* 2012).

In wine industry, amino-acids play an important in establishing several sensatory elements and are also involved in classification of wine as function of their variety. Amino-acids occurrence is from the grapes but they are consumed by easts during alcoholic fermentation that produce other types of substances as superior alcohols, aldehydes, esters and other volatile compounds. In the biological chain, the amino-acids are also produced by yeasts at the end of fermentation and released by autolysis in the wine, so they have an important role in establishing the final wine aroma (Robinson *et al.,* 2014).

In romanian wines, Bleiziffer *et al.*, 2017 determined the levels for amino acids in case of Fetească Albă variety and showed that the most important amino acids were glutamic acid, aspartic acid, gama-aminobutiric acid, alanine, glycine and lysine. Proline, as in major wine varieties is the main product released during fermentation. In this situation, the total content of free amino acids was 10.2 mg/ml

Scope of the study is to evaluate the levels of amino acids in case of four types of wine produced without sulfur dioxide.

## MATERIAL AND METHOD

*Materials and methods*

In terms of wines, a series of varieties were considered with $SO_2$ and without $SO_2$: Fetească Regală (S1) (S1'), Cabernet Sauvignon (S2) (S2'), Cabernet sauvignon rose(S3) (S3'), Fetească Regală Frizzante (S4) (S4') and were vinified by the traditional methods. All the varieties were vinified in vineyard Panciu.

Alanine (ALA), valine (VAL), leucine (LEU), isoleucine (ILE), proline (PRO), tryptophan (TRP), methionine (MET), serine (SER), arginine (ARG), histidine (HIS), threonine (THR), tyrosine (TYR), asparagine (ASN), glutamine (GLU), cysteine (CYS), aspartic acid (ASP), glycine (GLY), glutamic acid (GLM), lysine (LYS), phenylalanine (PHE) and valine (VAL) and were purchased from Sigma Aldrich -USA (min. 99,9%). For sample preparation, an AZ Fast Amino acid kit from Phenomenex – USA was used.

*Instruments*

Analysis was performed on a GC-MS from Shimadzu – Japan, consisting in a mass spectrometer with EI source QP2010 Plus. Gas chromatograph was Shimadzu GC2010 equipped with AOC -5000 autosampler. Zebron ZB AAA GC column with 10 m length, 0,25 mm internal diameter and 0,25 µm film thickness was used.

*Determination of amino acids*

Free amino acids were determined using a derivatization method based on Phenomenex EZfast application rules.

Gas Chromatography method used direct liquid injection of 2,0 µl in split mode at a split-ratio 1:15, at $250^{O}$ C. Carrier gas was helium at a flow rate of 1.1 ml/min in constant flow. The oven program started at 110°C, ramping at 30°C/min to reach 320°C, with a total run time of 15 minutes. Mass spectrometry settings included an MS source at 240°C and 7 kV, quadrupole at 180°C, and auxiliary temperature at 310°C. The scan range was 45–450 m/z with a sampling rate of 3.5 scans/s.

*Statistical analysis*

HSD test was used to separate mean values, with significance set at $p < 0.05$. Discriminant analysis (DA) further classified wines based on amino acid concentrations, variety, and sulfur dioxide content. All analyses were conducted using Statistica Software, version 14.0.

## RESULTS AND DISCUSSIONS

*Wine production*

Classical winemaking methods were applied, specific to each wine type. Wines with low sulfur dioxide involved varying doses of activators, antioxidants, and pectolytic enzymes. Representative fermentation values are shown in table 1.

Pectolytic enzyme levels were 3 g/hL using enzyme mixtures of pectinases, hemicellulases, cellulase, and protease. Gallic tannins were added to white and rosé wines, while catechin tannins were used for Cabernet Sauvignon. *Saccharomyces cerevisiae* was dosed within typical ranges (Cotea *et al.,* 2014), and for $SO_2$-free wines, *Oenococcus oeni* (0.7 g/hL) was included. Nutrients, as nitrogen sources were correlated with fermentation yeast levels.

*Table 1*

**Technological variations of the wines with $SO_2$ (S1, S2, S3, S4) and without $SO_2$ (S1', S2', S3', S4')**

| | S1' | S1 | S2' | S2 | S3' | S3 | S4' | S4 |
|---|---|---|---|---|---|---|---|---|
| **Tannins (g/hL)** | 120 | 34 | 69 | 25 | 54 | 13 | 17 | 5 |
| **Antioxidant (g/hL)** | 0 | 13 | 0 | 5 | 0 | 7 | 0 | 10 |
| **Pectolytic enzymes (g/hL)** | 3 | 2 | 3 | 3 | 3 | 3.3 | 2.5 | 1.5 |
| **Yeasts (g/hL)** | 22 | 15 | 21 | 29 | 49 | 18 | 19 | 18 |
| **Nutrient content (g/hL)** | 58 | 57 | 55 | 48 | 45 | 45 | 55 | 23 |

*Analysis of amino-acids*

Analysis of amino acids (figure 1) is based on the reaction of amino acids at room temperature with the addition of propyl chloroformate in basic media (Silva *et al.,* 2017). The reagents modified both the carboxyl and amino groups of the amino acids forming derivatives stable at room temperature for several hours. In these conditions the derivates have a corresponding polarity necessary to be well separated in method conditions. The following figure is representative to the spectrum of analyzed amino acids.

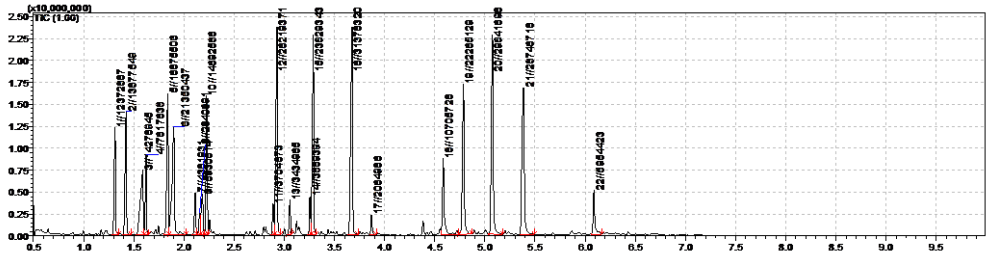

**Fig. 1.** Standard chromatogram for the analysis of aminoacids.

*Levels of amino acids and variation of the concentrations*

ALA showed significant differences in S3 samples ($p < 0.05$) compared to S1' and S4', both of which had lower $SO_2$ content. Similarly, GLY levels were higher in (S1) 14.08±2.58 mg/L and (S1') 10.94±2.45 mg/L (p=0.00047). Important decreases were registered with (S3') 13.25±8.23 mg/L also (S3) 4.03±0.55 mg/L (p=0.0005).

VAL levels decreased in the order S1 > S3 > S4 > S2, with $SO_2$-free forms following S1' > S3' > S2' > S4'. Notable differences occurred between S3' and S1 ($p = 0.004$) and between S3' and S1' ($p = 0.018$). LEU ranged from 2.67 ± 3.99 mg/L in S4 to 23.81 ± 18.75 mg/L in S1, with a 5 mg/L decrease in S2'. Some variations were noted between varieties regardless of $SO_2$ content. GLY also showed significant differences compared to S2' ($p = 0.0049$), S3' ($p = 0.0042$), and S4' ($p = 0.004$), all with low $SO_2$ content.

ILE had approximately equal concentrations, this amino acid was not affected by the treatment or the variety included in the study. The mean value of was 10.38 ± 0.11 mg/L.

THR presented higher levels for the classic winemaking forms (S4), (S3), and (S1). Most affected levels were recorded for (S3') 7.37±0.32 mg/L in relation with (S3) (10.13 ± 3.02 mg/L). THR showed much lower levels on the white wines, situation which was found in other studies (Garde-Cerdán, *et al.,* 2013). Thus, (S1) presented much lower levels for the forms with $SO_2$ and respectively without the addition of $SO_2$.

GLU maintained its levels regardless of the form of treatment. (S4) presented the highest levels 33.24±7.48 mg/L, continuing with (S1), (S2) and (S3). Regarding the forms without $SO_2$, the distribution was similar with

maximum concentrations of 24.07±4.77 mg/L, and the lowest concentrations were 18.57±0.89 mg/L.

PHE levels were highest in S3, with the strongest effect observed in $SO_2$-free S3' (5.88 ± 4.18 mg/L). In other $SO_2$-free varieties, decreases reached up to 2.08 ± 4.18 mg/L. LYS was abundant in S1 (41.28 ± 12.25 mg/L) and S3 (40.84 ± 10.34 mg/L), while lower levels were seen in $SO_2$-free S2' (25.28 ± 1.57 mg/L) and S3' (26.54 ± 1.86 mg/L).

HIS concentrations ranged from 8.22 ± 11.63 mg/L in S3' to 19.95 ± 7.49 mg/L in S1, with Cabernet Sauvignon varieties and rosé samples showing higher levels in $SO_2$-free forms.

TYR differed notably in S2 and S4; levels in $SO_2$-free S2' (12.70 ± 2.27 mg/L) and S4' (17.06 ± 5.26 mg/L) were slightly lower than their $SO_2$-treated counterparts. TRP showed significant differences, with 9.12 ± 6.84 mg/L in S3 versus 2.75 ± 2.66 mg/L in S3'.

PRO showed the most variability across varieties and $SO_2$ treatments, with S3 having the highest levels. $SO_2$-free S2' (1015.41 ± 191.84 mg/L) was notably higher than $SO_2$-treated S2 (924.09 ± 148.45 mg/L), with similar trends for S1' and S4'. However, PRO levels in S1' (444.79 ± 32.02 mg/L) and S2' were lower than in S4' or S3'.

A Discriminant Analysis on free amino acid content was conducted to develop discriminant functions, forming linear combinations of independent variables to distinguish categories of the variables assessed with significant group differences and classification accuracy. Sulfur dioxide content and wine variety were used as predictors (Pérez-Magariño *et al.,* 2013).

For sulfur dioxide, the analysis successfully differentiated groups, while for wine variety, the first two roots accounted for 52% of total variance. These roots created clear separation for Cabernet Sauvignon (S3/S3' and S4/S4'), whereas Fetească Regală (S1/S1') and Fetească Regală Frizante (S2/S2') showed close values on the negative side of the diagram (Figure 2).

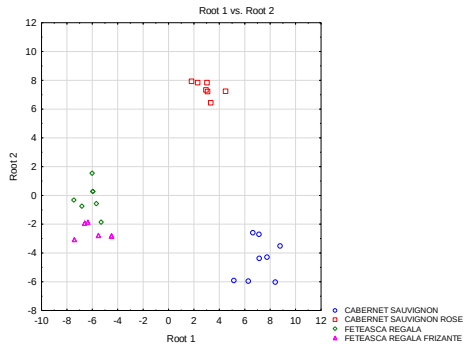


**Fig. 2.** Discriminant analysis for the varieties included in the study

## CONCLUSIONS

1. The level of amino acids proved to be specific to every variety. ILE and ASP showed constant values regardless of the variety or presence of sulfur dioxide as an additive. Some of the amino acids such as ALA, GLI, VAL, LEU, THR, LYS and SER have been severely affected in terms of lowering concentrations for low sulfur dioxide wines. In comparison, the amino acid levels were higher in the case of red / rosé wines compared to white wines.

2. When Tukey HSD test was applied, between the samples with $SO_2$ and without $SO_2$, statistical differences were obtained for ALA (Cabernet Sauvignon Roze), GLI (Cabernet Sauvignon Roze), PRO (Cabernet Sauvignon Roze, Fetească Regala Frizante)

3. Under these conditions, the discriminant analysis relieved differentiation on 2 sources only in case of variety as predictor. It was highlighted that parameters such as variety and differentiated treatment allow the individualization of wine types, regardless of the presence of sulfur dioxide added as an additive in the experiment.

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