BIOISOSTERS' IMPACT ON WINE CONDITIONING

IMPACTUL BIOIZOSTERILOR ÎN CONDIȚIONAREA VINURILOR

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Abstract. *Bioisosters are compounds with different substituents and with similar biological, physical or chemical properties. Pyridazine derivatives bioisosters are compounds with an intense biological activity, being used as anticancer, antituberculosis, antihypertensive, antifungal or antimicrobial agents. Several studies have tested the effect of some cyclic compounds with nitrogen in wine conditioning because stabilisation treatments against metal case are frequent operations used in winemaking production. The present paper aims to improuve the oenological products array used in wine conditioning, using pyridazine derivatives bioisosters, capable to expel the excess of metals, by complexing reactions. The impact of the tested compounds was studied on wines obtained from Fetească regală grape variety. The analysis method used was atomic absorption spectrometry.*
Key words: *wine conditioning, bioisosters, demethalytion.*

Rezumat. *Bioizosterii sunt compuși cu substituenți diferiți, dar cu proprietăți biologice, fizice și chimice asemănătoare. Derivații de piridazină sunt compuși cu o intensă activitate biologică, cu acțiune anticanceroasă, antituberculoasă, antihipertensivă, antifungică și antimicrobiană. Diferite studii au demonstrat efectul ciclurilor cu azot, în condiționarea vinurilor, împotriva casărilor metalice. Lucrarea propune lărgirea gamei de produse oenologine, prin utilizarea derivaților de piridazină bioisosterici, capabili să extragă excesul de metale din compoziția vinurilor, prin reacții de complexare. Impactul compușilor studiați a fost testat pe un vin alb, din soiul Fetească Regală. Analiza s-a realizat prin spectrometrie de absorbție atomică.*
Cuvinte cheie: *condiționarea vinului, bioizosteri, demetalizarea vinului.*

INTRODUCTION

Wine is a natural product and is one of the most widely consumed beverages [Zoecklein *et al*., 1994]. Due to its complex chemical structure, wine is regarded as a complex beverage [Han *et al*., 2022]. Wine develops complexity through the

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presence of chemicals derived from the grapes [Thorngate *et al*, 1997]. Trace elements in wine are important: organoleptic (Fe, Cu, Mn and Zn concentrations are directly related to the destabilization and oxidative evolution of wines) and toxicological (wine contamination with heavy metals may pose long-term human health risks and environmental damages, so toxic elements content should be under the allowable limit in wine identification) [Stafilov *et al.*, 2009; Sebecic *et al.*, 1998].

The content of metals in wine depends on various factors such as: natural sources (vineyard soil structure, climate, and geography), v*iticulture management practices (*fertilizers, pesticides, chemical sprays, grape-growing approaches) [Fiket *et al.*, 2011; Pohl, 2007; Stoleru *et al.*, 2015]; or c*ontamination during the winemaking process*, caused by prolonged contact with different materials: pipes, casks, steel tanks, fining and clarifying substances, equipment and other operations [Fiket *et al*., 2011].

The presence of excessive metal amounts induces wine denaturation with negative consequences regarding the smell, taste, and color, but also the appearance of the unavoidable wine turbidity [Moreno-Arribas *et al.*, 2009]. Today is a special interest for the content reduction of metals by using various treatments allowed by the legislation [Aceto *et al*., 2002; Conde *et al.*, 2002; De Lima *et al.*, 2004].

In this sense, the study offers information concerning synthesis and activity of three bioisosters (pyridazine derivatives, substituted with methyl group, fluorine atom and chlorine atom) in wine treatment. Bioisosterism is used to enhance the desired biological or physical properties of a compound without making significant changes in chemical structure. Also, bioisosterism is used to reduce toxicity, change bioavailability, or modify the activity of the lead compound and may alter the metabolism of the lead.

In literature, cyclic compounds with nitrogen, as imidazole and pyrrolidone ring are chelating agents and are a good choice in preventing denaturation of wine, by the insoluble salts that precipitate out [Moreno-Arribas *et al.*, 2009].

Pyridazine is a cyclic organic compound. The derivatives have demonstrated interesting potential applications in different fields of science, being highly valuable materials in medicinal chemistry, opto-electronics, agriculture etc. [Amariucai-Mantu *et al.*, 2021; Butnariu *et al.*, 2008]. So, these compounds have been extensively investigated being valuable compounds. Dates about synthesis and spectral analysis for pyridazine derivatives are in literature [Tucaliuc *et al*., 2013; Butnariu *et al*., 2018].

The purpose of this study is to decrease the metal content of wines obtained from *Fetească regală* grape variety by using the bioistosters that maintain and improve the quality of final product.

MATERIAL AND METHOD

Wine chemistry explains the flavour, balance, colour, stability that was once only possible through subjective description. Understanding the principles of wine chemistry

is very important for wine stability [Cotea *et al.,* 2009]. Undesirable, wine cases affect the clarification and/or stabilization and makes necessary the removal of insoluble and suspended products that may cause wine to become cloudy, gassy, form unwanted sediment deposit or tartric crystals [Robinson, 2006].

Cases of wines can be caused by protein aggregation, phenolic compounds or by several insoluble metal salts (only at concentrations higher than those usually found in wine). Also, the metal ions content from wine should not be over a specific quantity for not having a negative effect on the organism.

In literature, the methyl group (-CH_3), the fluorine atom (-F) and chlorine atom (-Cl) are presented as classical bioisosters and the pyridazine derivatives bioisosters have structures capable of extracting the metal ions from the wine, forming organic complexes, insoluble in wine. More than this, by filtering the treated wine, the used materials could be easily recovered – compared with the potassium ferrocyanide, where the cyanides remain as toxic residues.

To obtain the pyridazine derivatives bioisosters, pyridazine ring was treated with ω-bromacetophenone substituted in para position with classical bioisosters (the methyl group, the fluorine atom and chlorine atom) (Figure 1). The reactions take place mole by mole, in benzene anhydrous, in 3 hours at room temperature, by stirring. After filtered off and dried in vacuum, were obtained compounds (**5-7**) as crystals with good yield (η > 92 %). No purification required. They are stable in air and light and can be stored at room temperature for indefinite period of time. Structurally the compounds are similar. Only in para position from acetophenone group are different classical bioisosters: R = the methyl group (compound **5**), R = the fluorine atom or chlorine atom (compounds **6** and **7**).

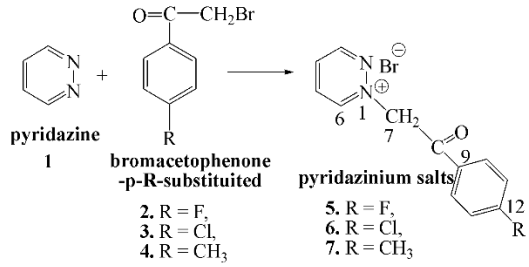

Figure 1. The synthesis of pyridazine bioisosters **(5-7)**

The structure of the compounds **(5-7)** was proved by spectral analysis: IR spectra, the 1H and 13C NMR spectra. These compounds have structures capable to extract the iron and copper ions from the wine, forming organic complexes, insoluble in wine. The main advantage is the possibility to recover used materials by filtering.

The study was performed on wine samples obtained from *Fetească regală* grape variety, according to the rules and regulations of the International Organization of Vine and Wine.

The wine samples (0.5 L) have been treated with 0,1 g from each tested product (**5**-**7**) and matrix solutions for the atomic absorption analysis were prepared at the same time, according to the Compendium of International Methods of Analysis – OIV, 2018. After 12 hours, the used compounds were recovered by filtering and their impact during the treatment was determined by atomic absorption spectrometry in flame, directly in wine. The addition of a spectral buffer (CsCl) to avoid ionisation of metals was necessary. The results were compared to the values of the control-sample.

RESULTS AND DISCUSSIONS

For both ions metal analyzed (iron and copper), standard solutions at different concentrations were prepared (Figure 2), according to the Compendium of International Methods of Analysis – OIV, 2018.

A known wavelength was selected, and the detector measured only the energy emitted at that wavelength. The concentration of the target atom in the sample increased and the absorption increased proportionally.

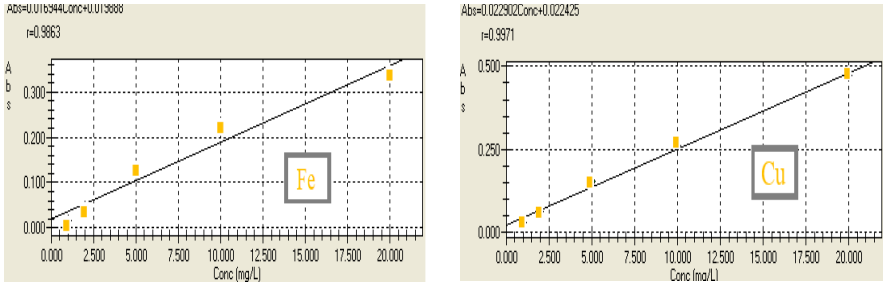

Figure 2: Calibration and standard curves for Fe and Cu

The comparative analysis of the obtained dates (**C_1 – C_3**) for wine samples treated with compounds (**5** and **7**) leads to the conclusion that the tested pyridazinic derivatives are not inert in wine, because the concentration in analyzed sample is less than in the control-sample. The results are presented in table 1.

Table 1

Atomic absorption results for Fe and Cu

Comp.	CS	C1	C2	C3
Conc. Fe (ppm)	12,9121	10,1012	9,8454	9,9085
Conc. Cu (ppm)	11,8142	9.784	9,8131	9,8001

C.S. = concentration for control sample

The compounds (**5 - 7)** extract easily iron and copper ions from the wine, by blocking metals in structures like *a cage*.

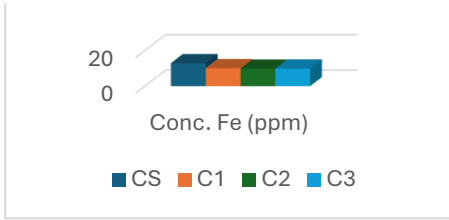

Figure 3: Iron ion concentration value

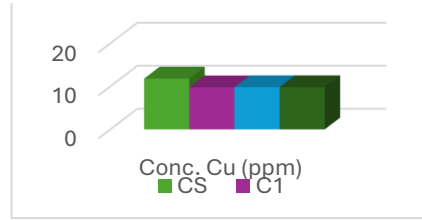

Figure 4: Copper ion concentration value

Figures 3 and 4 illustrate the decrease of trace metals (iron and copper) from wine composition, as a result of applied treatment with bioisosters (**5** – **7**).

The dates for the values obtained before treatment did not exceed normal limits allowed and were typical for the white wine obtained from *Fetească regală* grape variety.

CONCLUSIONS

For the first time, in this study have been used in wine conditioning pyridazinic bioisosters capable to absorb the metals by complexing reactions.

Atomic absorption spectrometry in flame is particularly suitable for direct identification and determination of trace metals in wine.

The comparative analysis of the obtained data leads to the conclusion that the tested pyridazinic bioisosters are not inert in wine: iron and copper ions were captured from the wine and the quality of wines was not affected.

The results shown that the bioisosters cand be used in winemaking for preserving, being a modern alternative to reduce or eliminate the trace metals.

The research will continue using compounds with similar structures.

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