THE DYNAMIC OF METHANOL AND ACETALDEHYDE IN WHITE WINES TREATED WITH SULPHUR DIOXIDE AND DIMETHYL DICARBONATE

DINAMICA METANOLULUI ȘI A ACETALDEHIDEI ÎN VINURILE ALBE TRATATE CU DIOXID DE SULF ȘI DIMETIL DICARBONAT

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Abstract: *Wine structure is very complex, consequently, the latest researches has been focused on the detailed analysis of this beverage. One of the most important compounds that can be found in wines is methanol. The toxicology of methanol and the admissible limits established by OIV, especially the effect on consumer's health, have to be considered. In this sense, the maximum permissible quantity of methanol and changes in its composition in wines must always be discussed.*
For this study, forty-five samples were obtained from a blend of Fetească regală and Muscat Ottonel grape varieties at the experimental wine cellar of the Oenology Laboratory of the Faculty of Horticulture from Iași. All variants were treated with 6 % SO_2 solution (40, 80, 160 mg/L) and dimethyl dicarbonate liquid solution, in various ratios (0, 100, 200 µL/L).
Also, in this experiment, yeasts such as Schizosaccharomyces spp. and Brettanomyces spp. were inoculated separately (S-Schizosaccharomyces pombe, B-Brettanomyces bruxellensis) and the evolution of methanol content and acetaldehyde in wines was recorded following the administration of treatments with dimethyl dicarbonate and sulphur dioxide.
The main purpose of this research is to evaluate the methanol content in wine samples using a gas chromatography method and the possible transformation in wine composition (methanol concentrations) produced by DMDC. Moreover, an important compound that can be formed in wines due to SO_2 presence is acetaldehyde, so, the quantity and the effect on wine composition were discussed.
The results show different amounts of methanol and acetaldehyde due to the reactions of the used treatments with specific compounds of wine. The concentration of methanol and acetaldehyde in the studied wine samples are within permitted limit for white wines, 250 mg/L methanol.
Keywords: methanol, acetaldehyde. sulphur dioxide, dimethyl dicarbonate, yeasts

Rezumat: *Structura vinului este foarte complexă, prin urmare, ultimele cercetări s-au concentrat pe analiza detaliată a acestei băuturi. Unul dintre cei mai importanți compuși care pot fi găsiți în vinuri este metanolul. Trebuie punctate aspectele referitoare la toxicologia metanolului și limitele admise*

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stabilite de OIV, în special efectul asupra sănătății consumatorului. În acest sens, cantitatea maximă admisă de metanol și modificările produse în vinuri trebuie întotdeauna discutate.

Pentru acest studiu, au fost obținute patruzeci și cinci de probe dintr-un cupaj obținut din soiurile Fetească regală și Muscat Ottonel, în cadrul cramei experimentale a Laboratorului de Oenologie al Facultății de Horticultură din Iași. Toate variantele au fost tratate cu soluție de SO_2 6% (40, 80, 160 mg/L) și soluție lichidă de dimetildicarbonat, în diferite concentrații (0, 100, 200 mg/L).

De asemenea, în acest experiment, levuri precum cele din genurile Schizosaccharomyces spp. și Brettanomyces spp. au fost inoculate separat (S-Schizosaccharomyces pombe, B-Brettanomyces bruxellensis) în diferite concentrații (0, 30, 100 ufc/mL) iar evoluția conținutului de metanol și acetaldehidă în vinuri a fost înregistrată în urma administrării tratamentelor cu dimetildicarbonat și dioxid de sulf.

Scopul principal al acestei cercetări este acela de a evalua conținutul de metanol din probele de vin folosind o metodă de cromatografie gazoasă și posibila transformare a compoziției vinului (concentrații diferite de metanol) produse în urma tratamentului cu dimetildicarbonat. Mai mult, un compus important care se poate forma în vinuri datorită prezenței dioxidului de sulf este acetaldehida, astfel încât cantitatea și efectele asupra compoziției vinului au fost discutate.

Rezultatele indică cantități diferite de metanol și acetaldehidă datorită reacțiilor cu compușii specifici ai vinului apărute în urma tratamentelor administrate. Concentrația de metanol și acetaldehidă în probele de vin studiate se încadrează în limita permisă pentru vinurile albe, și anume 250 mg/L metanol, respectiv 125 mg/L acetaldehidă.

Cuvinte cheie: metanol, acetaldehidă, dioxid de sulf, dimetildicarbonat, levuri.

INTRODUCTION

Wine represents a complex system with important components that contributes to its final quality. Also, it is recognized as the most consumed and studied beverage from the last years, its consumption being influenced by the consumer's lifestyle. Therefore, in order to ensure consumer's health, the wine parameters must be strictly controlled, especially methanol and acetaldehyde content (before and during fermented-drink production) (Yong Sheng-Li, 2018), sulphur dioxide and dimethyl dicarbonate levels. To observe the wine quality evolution, it is necessary to quantify some physical or compositional parameters that define the main organoleptic properties (Avramescu, 2002).

Methanol, known as methyl alcohol, is a colourless liquid with characteristic odour, miscible in water, with boiling point 65°C and melting point -98°C (FIVS, 2016). It is produced before and during alcoholic fermentation from the hydrolysis of pectin by pectinase enzymes - methyl pectin esterase which are naturally present in fruits, more methanol being produced when must is fermented on grape skins (generally more in red wines than in *rosé* or white wines) (Methanol in Wine - FIVS - 2016-08-22, 2/7).

The methanol content in wines is strictly regulated by the International Organisation of Vine and Wine (OIV) at < 400 mg/L for red wines and for white or *rosé* wine < 250 mg/L (OIV, 2019). The amounts of methanol in wine depend on several factors such as: grape variety (the grape skins which contain a high content of pectins) (Ribereau-Gayon *et al.*, 2006) and health, fermentation temperature, maceration treatment and different applied treatments in winemaking process (Cabaroglu, 2005).

Acetaldehyde, also known as ethanal, represents an important volatile carbonyl compound that can be formed in wine through the yeasts activity (biologically) or by wine oxidation (chemically) (Nykanen, 1986). It is particularly reactive and can react with amino acids to produce several flavour compounds (Griffith and Hammond, 1989). In small quantities produces herbaceous, green grass, fruity or nutty aromas and excess acetaldehydes produces irritation and unpleasant odours (Miyake and Shibamoto, 1993).

Modern researchers focused on the actions of oenological products that can improve the wine quality, composition, physical chemical parameters, colour and stability. One of the most common and useful substance with a complex role in winemaking is sulphur dioxide. It is used as a preservative product due to its antioxidant and antimicrobial role (it inhibits the growth of yeasts and bacteria) (Reynolds, 2010). Also, it inhibits the enzymatic or non-enzymatic browning reactions during the production process and storage. It is well known that small amounts of SO_2 are naturally produced by yeasts, influencing the chemical structure of wine and its stability (Pati, 2014; Ribereau-Gayon, 2006).

Sulphur dioxide is usually used for stopping alcoholic fermentation for producing beverages with residual sugar content. However, SO_2 causes a metabolic change in active yeast leading to the formation of acetaldehyde and resulting in higher SO_2 requirements in the final product (Erhu-Li, 2020). Although it is known that SO_2 controls wine quality, it was also found that the variable levels of SO_2 have a direct influence on the acetaldehyde levels produced during fermentation process (Francois, 2020).

Commission delegated regulation (EU) 2019/934 indicates the following maximum limits to be respected: 150 mg/L for red wines; 200 mg/L for white ones and up to 350 mg/L total SO_2 for sweet wines.

Modern oenology focus on a complex product which was used for the beginning in juices and now it is considered useful in wines. Dimethyl dicarbonate or DMDC is recognised as being an effective preservative and antimicrobial substance for low alcoholic wines, particularly for those with residual sugar content. It is a colourless liquid with a sharp odour depending on numerous factors such as, wine physical-chemical parameters (pH, ethanol, sugar content), used yeasts, storage conditions (Bartowsky, 2009; Costa *et al.*, 2008). This product has been tested as an alternative of SO_2 in winemaking (Divol *et al.*, 2005) and approved on a maximum of 200 mg/L dose in wines that contain more than 5 g/L of residual sugar (Regulation (EC) No 643/2006).

There are numerous yeast species related to winemaking, particularly non-*Saccharomyces*, that deserve special monitoring due to their great potential of determining certain changes in wine composition. *Schizosaccharomyces pombe*, also known as fission yeast, was discovered by Lindner, in 1983. It has certain disadvantages such as its low fermentation speed or the development of undesirable flavours and aromas (Loira, 2018).

A particularity of *S. pombe* is that it can grow in environments with low water activity (it is an osmophilic yeast), therefore it can be found in media with high sugar content (Kurtzman, 2010). Also, it can develop in very low pH environments, in a varied range of temperatures, being resistant to food and beverage preservatives such as: sulphur dioxide, dimethyl dicarbonate, benzoic acid and actidione (Suárez-Lepe, 2012; Escott, C., 2017; Loira, 2018).

The *Brettanomyces / Dekkera* yeasts can be found in fermenting must and in wines, typically grow in low cell numbers after alcoholic and malolactic fermentation during wines storage. It is recognized because of the ‘bretty’ flavors it imparts, described as smoky, barnyard, plastic, burnt plastic, horse sweat, wet wool, leather (Henick-Kling, 2000).

The main purpose of this research was to analyze the effect of different stabilization treatments, with DMDC and SO_2on the volatile compounds of white wines obtained from a blend of Muscat Ottonel and Fetească regală grape variety.

MATERIAL AND METHOD

Winemaking procedure. Laboratory analyses were realized according to the International Organization of Vine and Wine methods (OIV, 2019).

For this experiment, Muscat Ottonel and Fetească Regală grapes were manually harvested at full maturity in autumn of 2018 from Iași vineyard. For the processing of raw materials (grapes) the classic vinification method for obtaining white wines was used.

After the reception, destemming, crushing and pressing follows, the juice was transferred in a stainless tank for fermenting phase and *Brettanomyces bruxellensis* and *Schizossacharomyces pombe* yeasts were inoculated in different amounts (30, 100 ufc/L).

After this step, obtained wine was divided in three aliquots in which different concentration of sulphur dioxide (40, 80, 160 mg/L) were added.

The resulted mixture was filtered using sterile filters, bottled into 750 mL glass bottles and then different amounts of dimethyl dicarbonate (100 or 200 mg/750 mL) were added. Bottles were stored under controlled temperature conditions until gas chromatography analyses were performed.

Gas chromatograph procedure. To identified the volatile compounds in experimental samples an Agilent gas chromatograph was used. Working conditions depend on the complexity of the mixture to be analyzed and most often on the characteristics of the chromatographic column on which the separation is performed.

RESULTS AND DISCUSSIONS

The volatile compounds identified in the experimental samples are acetaldehyde and methanol, known in oenology with an impact especially in chemical composition and sensorial parameters of wines.

The increase in methanol content in the analyzed samples is due to the hydrolysis of DMDC into methanol and carbon dioxide. Experimental samples registered values between 51.07 mg in P25 sample treated with 80 mg/L SO_2 without DMDC and 148.82 mg in P39 sample treated with 160 mg/L SO_2 and 200 mg/L DMDC. However, the concentrations resulted after the administration of this treatments are within the permissible limit in white wines to 250 mg/L methanol and not represent a problem for a human health.

Table 1

Experimental samples of whitewines

P1	40 S-0 L-0 DMDC	P16	80 S-0 L-0 DMDC	P31	160 S-0 L-0 DMDC
P2	40 S-0 L-100 DMDC	P17	80 S-0 L-100 DMDC	P32	160 S-0 L-100 DMDC
P3	40 S-0 L-200 DMDC	P18	80 S-0 L-200 DMDC	P33	160 S-0 L-200 DMDC
P4	40 S-30 B-0 DMDC	P19	80 S-30 B- 0 DMDC	P34	160 S-30 B-0 DMDC
P5	40 S-30 B-100 DMDC	P20	80 S-30 B-100 DMDC	P35	160 S-30 B-100 DMDC
P6	40 S-30 B-200 DMDC	P21	80 S-30 B-200 DMDC	P36	160 S-30 B-200 DMDC
P7	40 S-100 B-0 DMDC	P22	80 S-100 B-0 DMDC	P37	160 S-100 B-0 DMDC
P8	40 S-100 B-100 DMDC	P23	80 S-100 B-100 DMDC	P38	160 S-100 B-100 DMDC
P9	40 S-100B-200DMDC	P24	80 S-100 B-200 DMDC	P39	160 S-100 B-200 DMDC
P10	40 S-30 Schiz.-0 DMDC	P25	80 S-30 Schiz.-0 DMDC	P40	160 S-30 Schiz.-0 DMDC
P11	40 S-30 Schiz.-100 DMDC	P26	80 S-30 Schiz.-100 DMDC	P41	160 S-30 Schiz.-100 DMDC
P12	40 S-30 Schiz.-200 DMDC	P27	80 S-30 Schiz.-200 DMDC	P42	160 S-30 Schiz.-200 DMDC
P13	40 S-100 Schiz.-0 DMDC	P28	80 S-100 Schiz.-0 DMDC	P43	160 S-100 Schiz.-0 DMDC
P14	40 S-100 Schiz.-100 DMDC	P29	80 S-100 Schiz.-100 DMDC	P44	160 S-100 Schiz.-100 DMDC
P15	40 S-100 Schiz.-200 DMDC	P30	80 S-100 Schiz.-200 DMDC	P45	160 S-100 Schiz.-200 DMDC

S - Samples treated with different concentrations of sulphur dioxide (40, 80, 160 mg/L)
DMDC - Samples treated with different concentrations of dimethyl dicarbonate (100, 200 mg/L)
B - Samples inoculated with yeasts of *Brettanomyces bruxellensis*
S - Samples treated with yeasts of *Schizossacharomyces pombe*

Samples treated with different concentrations of sulphur dioxide 40,80,160mg/L and with or without DMDC registered minimal values of acetaldehyde concentration, which shows that treatments did not have a negative effect on the experimental samples. In this experiment, values between 4.37 mg/L in P2 sample treated with 40 mg/L SO_2 and 100 mg/L DMDC and 27.93 mg/L in P23 sample treated with 80 mg/L SO_2 and 100 mg/L DMDC were obtained. In small quantities, acetaldehyde has positive aspects, it can produce herbaceous notes in freshly fermented wine.

Comparative graphics between methanol and acetaldehyde concentrations of samples treated with different concentration of sulphur dioxide and DMDC

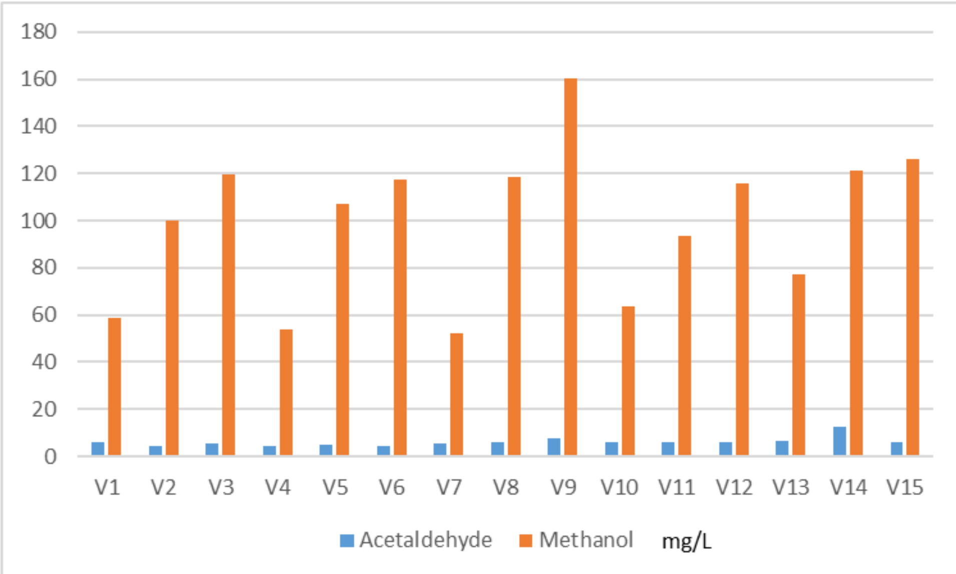

Fig. 1 Samples treated with 40 mg/L SO_2 and DMDC

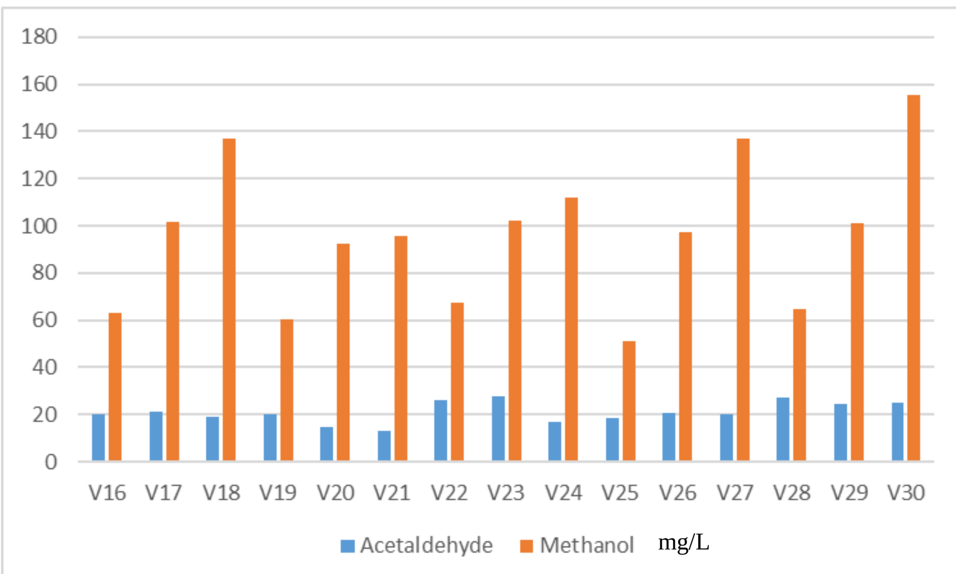

Fig. 2 Samples treated with 80 mg/L SO_2 and DMDC

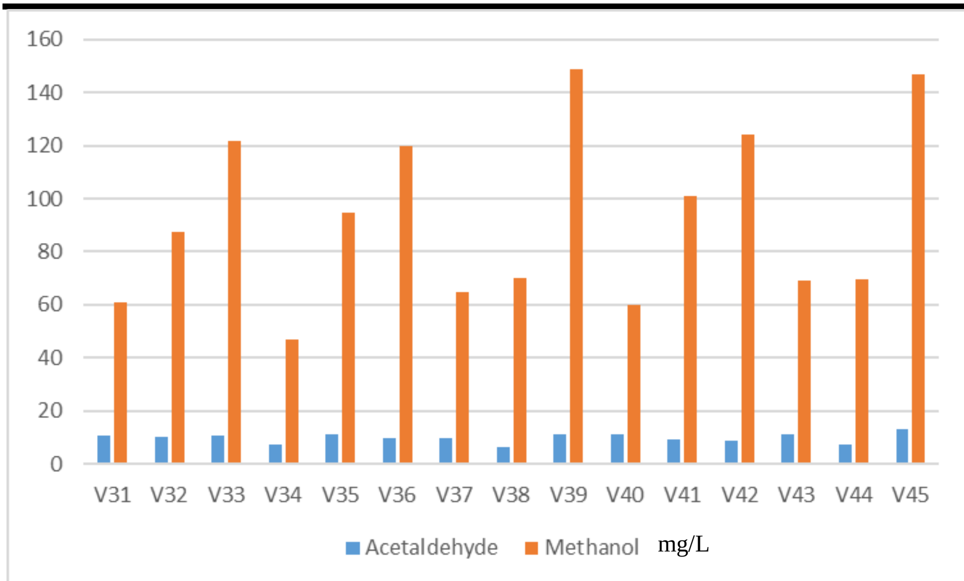

Fig. 3 Samples treated with 160 mg/L SO_2 and DMDC

CONCLUSIONS

Conditioning treatments used in winemaking act differently, separately, or together, determining some changes in wines composition.

This research confirms that sulphur dioxide and dimethyl dicarbonate contribute positively to some modifications in wines, the concentrations of methanol and acetaldehyde identified registered values, all within the limits mentioned by OIV legislation.

This stabilization treatment can be a modern alternative for winemakers, due to the synergetic activity between SO_2 and dimethyl dicarbonate, that contributes to improve quality of wines, especially determining a good microbiological stabilisation.

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