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EFFECT OF ADDED SULPHUR DIOXIDE LEVELS ON THE AROMA CHARACTERISTICS OF WINES FROM PANCIU WINE REGION

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Abstract. In the wine-making process, the yeasts form over 20 higher alcohols that improve the sensation of palatine veil for the wine. The amount of higher alcohols is lower for white and rosé wines obtained from clarified musts fermented at moderate temperatures. The formation of higher alcohols is confirmed by the fact that the addition of certain nitrogen compounds to a glucose-containing culture medium leads, after the fermentation, to an increase in the higher alcohol content. Data evaluate by comparison the levels of higher alcohols, esters and aldehydes, important to fermentation process in case of several wines from Panciu region. The wine-making was done by classical methods and by using specific protocols for low content sulphur dioxide. This study is focused to compare wines obtained from two grape varieties produced at industrial level: Feteasca regala vs. Feteasca regala Frizzante and Cabernet Sauvignon rosé vs. Cabernet Sauvignon. The compounds were analyzed by GC-FID method with head-space technique for methanol, 1-propanol, 2-methyl-propanol, 1-butanol, ethyl acetate, ethyl lactate, isoamyl acetate and acetaldehyde. The results showed that in almost every comparative study the levels of aroma compounds are maintained, when compared with the wines with low content of sulphur dioxide. Tuckey test showed that 1-propanol and ethyl acetate register statistically significant differences for Cabernet Sauvignon rosé and for Feteasca regala Frizzante.

Key words: Higher alcohols; GC-FID; Fermentation; Sulphur dioxide.

INTRODUCTION

Wine is produced by exploiting the metabolism of several yeast strains. In these conditions, indigenous microorganisms grow in fermentation media and influence the yeast and bacteria that can also produce several unwanted metabolites as higher alcohols, acetic acid and acetaldehyde (Sharma, R. et al. 2020). In the wine making practices the addition of sulphur dioxide prevents the oxidation processes and also minimizes the grows of viable microbiological organisms, which are of major importance in wine production and aging. Sulphur dioxide has the role to inhibit the abundance of several yeast strains, in this way some aroma characteristics are denatured and the wine could have different characteristics (Morgan, S. et al. 2019). For instance, the acetic acid had higher levels than ethyl and isoamyl acetate in the wines with low content of sulphur dioxide than in the wines treated with sulphur dioxide 60 mg/L. This showed the potential negative impact upon wine characteristics due to the presence of acetic bacteria. On the other hand, an excess of sulphur dioxide produces diacetyl compounds which affect in a larger measure the quality of wines (Sun, Y. et al. 2016).

The sulphites have antimicrobial and antioxidant properties, but they have a toxic potential and may be also allergens for the consumers. The wines with the content of sulphur dioxide higher than 10 mg/L should be mandatorily labelled ”contain sulphite” or the message have to be stated on the wine label. Because of the new regulations, there was a continuous concern regarding the use of lower content of this additive in wine production. For this purpose, some modifications in the wine production protocols were tested in order to have the same results in grape treatment, fermentation, stabilization, correction, filtration and aging (Nardini, M. et al. 2018).

Regarding the wine aroma, there are two main characteristics which define the typicity of the final products. From this category, four esters are involved, such as isoamyl acetate (ISA), ethyl lactate (ETL), ethyl acetate (ETA) and 5 alcohols as *n*-propanol (1PROH), *n*-butanol (1BUTOH), isobutanol (2M-1PROH), isopentanol (2M-1BUTOH) and 2-phenetanol (2PHET). These compounds were monitored in the present study in wines with sulphur dioxide as additive and wines without SO_2 added.

MATERIALS AND METHODS

In terms of wines, a series of grape varieties cultivated in the vineyard Panciu were considered with SO_2 and without SO_2: Feteasca regala (S1), (S1’), Cabernet Sauvignon (S2), (S2’), Cabernet Sauvignon rosé (S3), (S3’), Feteasca regala Frizzante (S4) (S4’) and were processed by traditional methods.

The study included a series of alcohols, acetaldehyde and esters such as acetaldehyde, methanol, butan-1-ol, butan-2-ol, 1-propanol, 2-methyl-propan-1-ol, 2-methyl-pentane-2-ol, isoamyl acetate and ethyl lactate that were purchased from Sigma Aldrich and had a concentration of minimum 99.9%.

The analysis was performed on a gas chromatograph with flame ionization detector GC-FID, Agilent 7890B from Agilent Technologies equipped with a GC sampler 80. Head-space technology was used and a Phenomenex Zebron FFAP GC column of 50 m length, 0.32 mm internal diameter and 0.5θ μm film thickness.

For head space injector, incubation temperature was maintained at 85°C for 15 minutes, while the syringe temperature was of 87°C, and a volume of 1,5 mL was injected in the split/splitless injector, which operated at a split-ratio 2:1, at 250 °C.

The gas chromatograph used carrier hydrogen at a flow rate of 1.6 mL/min. Oven program started at 30°C for 5 min, then 2 temperature ramps were applied. Firstly, an increase of 2.3°C/min up to 80°C and a secondary ramp of 25°C/min up to 250°C. Finally, a hold time of 3 minute was maintained. The total chromatography time was of 45 minutes. The flame ionization detector had a temperature 250 °C and an acquisition rate of 20 Hz.

Statistical analysis

Wine variety and sulphur dioxide as additive were used as variables for data analysis in order to evaluate the variance in case of aroma compounds. The mean value separation was evaluated by Tukey's Honest Significance Difference (HSD) test.

RESULTS AND DISCUSSIONS

General procedures of the wine making process followed the classical methods but with slight modifications for the low sulphur dioxide varieties. The wine-making program started with grapes protection. For the sulphur dioxide wines, the sulfiting was performed by adding potassium metabisulphite, ascorbic acid and gallic tannin. Instead, for the lots intended to be produced without SO_2 a special strain *Pichia kluyveri* was included. The fermentation step used *Saccharomyces cerevisiae* yeast at a dose of 20-30 g/hL and specific nutrients. The maintenance of wine on lees was performed on carbon dioxide protection until bottling. Prior to bottling, the wines were subjected to tartaric-stabilization using bentonite 1 g/L, silicate and polyvinylpyrrolidone 10-20 g/L. Several corrections were performed for wines without sulphur dioxide using lactic acid and gallic tannin, while for wines with SO_2, additive correction of acidity and correction of sulphur dioxide up to 50 mg/L SO_2 free were performed. Malolactic fermentation was done in conjunction with alcoholic fermentation, by monitoring the lactic acid content for all the wines without sulphur dioxide and all types of red wines.

The determination of aroma compounds

The method ensured the separation of all the compounds, with a minimum resolution of at least 2 being achieved for every compound. The chromatography time of 45 minutes was necessary due to the complexity of the samples and in order to confirm the resolution by mean of standard addition that showed a high chromatographic profile, as showed in the Figure 1.

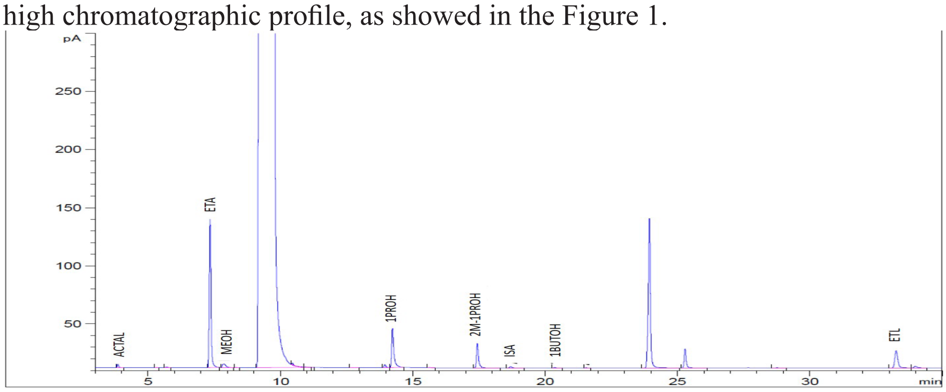

Figure 1. *CG chromatogram for Feteasca regala Frizzante with SO_2 (S4) (ACTAL (acetaldehyde) – (Rt-3.86); ETA (ethyl acetate)– (Rt-7.35); MEOH (methanol)– (Rt-7.87); 1PROH (n-propanol) – (Rt – 14.24); 2M-1PROH (isobutanol) – (Rt – 17.45); ISA (isoamylacetate)– (Rt – 18.71); 1BTOH (n-butanol) – (Rt-20.36); ETL (ethyl lactate) – (Rt-33.27)*

The average values of alcohols, esters and acetaldehyde are presented in Table 1 by comparing representative samples for the varieties included in the study. The comparison is performed in parallel on specimens with sulphur dioxide, for which the level of free SO_2 was established at 50 mg/L and samples without SO_2 with levels of free form up to 5 mg/L.

Table 1. *Average values of aroma compounds for wine varieties included in the study*

	ETL	ACTAL	ETA	MET	1PROH	2M-1PROH	ISA	1BUTOH
S2	178.28	4.05	88.44	137.61	10.83	28.10	1.72	1.44
S2’	229.52	0.61	122.53	152.29	15.39	23.38	1.21	1.33
S3	339.53	0.95	70.17	21.30	50.23	7.10	1.09	1.17
S3’	269.02	2.98	81.66	23.74	13.49	8.95	1.37	1.05
S4	155.83	2.71	54.97	33.26	36.96	8.82	1.87	0.84
S4’	257.31	3.86	39.17	29.27	23.34	23.17	7.72	2.35
S1	154.22	4.86	46.28	40.25	23.93	8.74	2.39	0.81
S1’	201.60	4.45	35.80	36.71	26.76	9.80	1.55	0.70

Note: (S2 - Feteasca regala + SO_2, S2’- Feteasca regala – SO_2; S1- Cabernet Sauvignon + SO_2; S1’- Cabernet Sauvignon – SO_2, S3 - Cabernet Sauvignon rose + SO_2, S3’- Cabernet Sauvignon rose – SO_2, S4 - Feteasca regala Frizzante + SO_2, S4’– Feteasca regala Frizzante – SO_2

The maceration of the solid parts of the grape and skins in the must, which have more pectolytical substances than the juice, leads to the production of wines with a high methanol content (Martínez-Pérez, M.P. et al. 2020). In red wines, methanol has higher values for Cabernet Sauvignon (144.95 mg/L). Regarding the use of pectolytic enzymes which had doses of 3 g/hL for Cabernet Sauvignon, Cabernet Sauvignon rosé and Feteasca regala, and lower doses for Frizzante variety, there was no significant correlation between the doses used and the level of methanol in the analysed samples.

The formation of several alcohols is the result of intermediate products derived from the degradation of carbs during the fermentation. In this situation, 2M-1PROH may be the result of biotransformation of valine. Another study showed that VAL had lower values than typical doses of 50 mg/L for white wines, 50 mg/L for red wines and 100 mg/L for frizzante wines. Although, the coefficient of the correlation between the valine levels and the level of 2M-1PROH was of only −0.2809, showing that the transformation of valine in 2M-1PROH was not highly correlated.

As for the the category of higher alcohols, the levels of 2M-1PROH were higher for Cabernet Sauvignon (S2) 28.1 mg/L, while a lower value was registered for Feteasca regala Frizzante (S3) with concentrations of 7.1 mg/L for the varieties with SO_2. On the contrary, higher values for Feteasca regala Frizzante without sulphur dioxide (S4’) 23.17 mg/L with significant difference (p=0.044) against the form free of SO_2 were registered. For the other varieties, the levels of 2M-1PROH were close, Cabernet Sauvignon rosé had an average value of 8.03 mg/L and Feteasca regala 9.27 mg/L.

1-butanol (1BTOH) had lower levels than other alcohols, with average concentrations between 1.59 mg/L for Feteasca regala Frizzante and 0.76 mg/L for Feteasca regala (classic method). There were no significant differences between the varieties in relation with the content of sulphur dioxide, the only exception was recorded by Feteasca regala Frizzante with values of 2.35 mg/L for the sample without SO_2 (S4’) and 0.84 mg/L for the sample with SO_2 (S4) (p=0,0017).

The use of selective yeasts maintained the formation of ACTAL to levels less than 5 mg/L in wines (Vashakidze, P., Bezhuashvili, M. 2020). The formation of ACTAL is stimulated by the sulphur dioxide in must. On the other hand, the formation of this compound is promoted by the oxidation of the ethylic alcohol. In the case of Cabernet Sauvignon with SO_2 (S2) the levels of ACTAL were higher 4.05 mg/L than in the case of the wine without SO_2 (S2’) 0.61 mg/L. It was in contrast with the other varieties which had higher values without sulphur dioxide, the fact explained by the possibility of bounding to sulphurous acid produced by sulphitation of wines (Zara, G., Nardi, T. 2021).

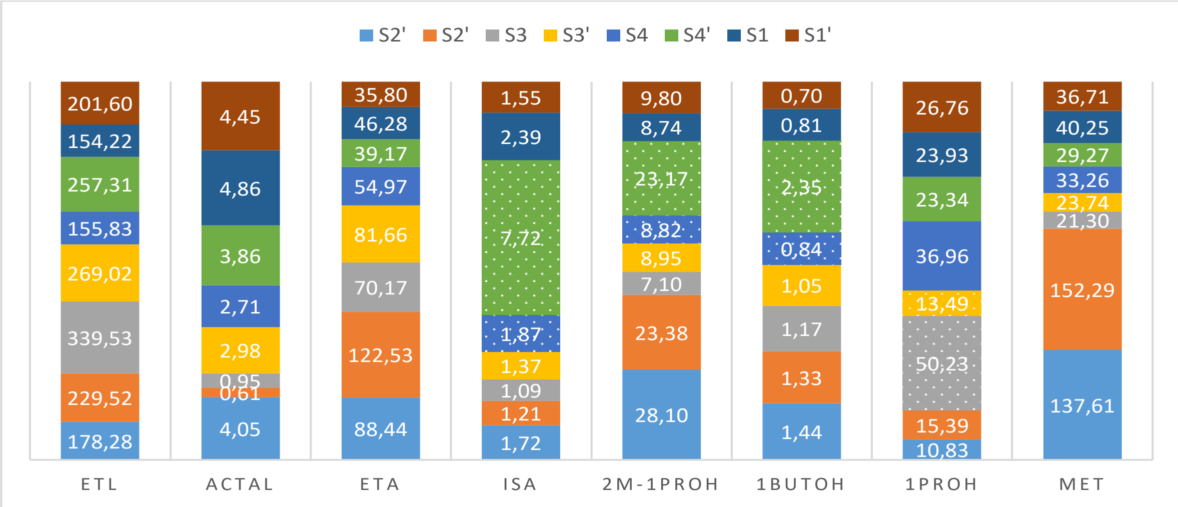

Figure 2. *Comparative evaluation of the aroma compounds (isoamyl acetate (ISA), ethyl lactate (ETL), ethyl acetate (ETA), n-propanol (1PROH), n-butanol (1BUTOH), isobutanol (2M-1PROH) for the varieties included in the study. Hatched areas correspond to level that had statistically significant difference ($p<0.05$)*

In direct relation with acetaldehyde, the concentration of ethyl acetate (ETA) was correlated for wines without SO_2. The correlation coefficient was of (−0.91), but in case of wines with SO_2 as additive the variation between the two compounds could not be correlated. The levels of ETA reached the highest level for Cabernet Sauvignon without SO_2 (S2') - 122.53 mg/L, although for the SO_2 form (S2) the level was of 88.44 mg/L. Cabernet Sauvignon rosé without SO_2 (S3') also recorded higher levels of 81.66 mg/L and 70.17 mg/L for the variety with SO_2 (S3). Instead, the white varieties of Feteasca regala recorded lower levels and the concentrations of forms without SO_2 (S1') were inferior compared with the samples with SO_2 as additive (S1) (Figure 2). Regardless of the treatment, the differences were not statistically significant, therefore wine quality was not affected by the absence of sulphur dioxide.

The ethyl lactate (ETL) had different values of concentration in relation with the varieties included in the study. Higher values compared with other esters are explained by the fact that the wines without sulphur dioxide and the red wines had malolactic fermentation (Arapitsas, P. et al. 2018). Regarding the wines without sulphur dioxide, the values of ETL are higher than the values for wines with SO_2 (Feteasca regala (S4) (155.83 mg/L) and Feteasca regala Frizzante (S1') (201 mg/L)), also for Cabernet Sauvignon (229.52 mg/L) (S2'). Cabernet Sauvignon rosé recorded a different behaviour because of the lower value for the wine without SO_2 (S3') (269.02 mg/L) in relation with the wine which had sulphur dioxide as preservative (S3) (339.53 mg/L). Literature reported values between 5 – 8 mg/L for ETL in case of wines without malolactic fermentation (Lasik-Kurdyś, M. et al. 2018), but the levels found in this experiment could be explained by the acidity correction with several doses of lactic acid, therefore the high values of ETL can be associated with the content of lactic acid introduced in final step of winemaking.

The lowest values were obtained for the ISA, the level of concentrations being between 1.21 mg/L for Cabernet Sauvignon (S2') and 7.72 mg/L for Feteasca regala Frizzante (S4). These levels are explained because of the stabilization of esterification equilibria, which gave hydrolysis reactions or chemical reactions with acids (Makhotkina, O., Kilmartin, P. 2012). The presence of ISA was not influenced by the sulphur dioxide in wines, the only difference is given by Feteasca regala Frizzante, which had values for ISA of 1.87 mg/L for the variety with SO_2 (S4) in relation with the sample without the additive (S4'). The evaluation showed a significant difference, as the comparison based on Tuckey test *p* resulted in a value less than 0.05.

1-propanol (1PROH) had values between 50.23 mg/L for Cabernet Sauvignon rosé with SO_2 (S3) and 13.49 mg/L for the samples without SO_2 (S3') ($p<0.05$). This was the only variety that was affected in terms of concentrations of 1PROH. Some slight variations were also recorded by Cabernet Sauvignon, but 1PROH was not affected in the same way as the rosé variety (10.83 mg/L (S2) and 15.39 mg/L (S2'). As for the white varieties, the values were closer so no effect was registered, the 1PROH had close values in terms of concentrations.

CONCLUSIONS

The level of some aroma compounds proved to be specific depending on each variety. Acetaldehyde, ethyl acetate, 1-butanol and ethyl lactate showed constant values. For the varieties without sulphur dioxide the values were slightly higher, so the wines maintained their typical aroma.

When Tukey HSD test was applied, the samples with SO_2 and without SO_2 registered statistical differences for 1-propanol (Cabernet Sauvignon rosé), 2-methylpropan-1-ol, isoamyl acetate and 1-butanol. The latest three compounds varied for regala Frizzante, the variety that had the most significant variations, but in this case the values for the samples without sulphur dioxide were higher.

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