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PRELIMINARY STUDY ON THE INHIBITION OF ALCOHOLIC FERMENTATION USING OCTANOIC AND DECANOIC ACIDS TO OBTAIN AROMATIC WINES WITH RESIDUAL SUGAR

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Abstract

Aromatic wines produced from 'Tămăioasă românească' variety can be fermented to dryness, but they are usually preferred with residual sugar. To preserve some of the sugar unfermented the natural alcoholic fermentation can be stopped by several methods. One of the newest methods researched is the use of medium chain fatty acids, which are naturally produced by yeasts and are also inhibitors of fermentation. In this study doses of 10-30 mg/l of octanoic and decanoic acids, as well as combinations of both acids in doses of 15 mg/l were used to inhibit the fermentation of wines inoculated with 2 different yeast strains. The final sugar content of the resulted wines along with some other physico-chemical and sensory parameters were determined and compared. Our preliminary observations showed that the inhibition is dose dependent and also that the decanoic acid tends to be more inhibitory than octanoic acid.

Key words*: 'Tămăioasă românească' wine, alcoholic fermentation inhibition, medium chain fatty acids, octanoic acid, decanoic acid.*

INTRODUCTION

Sweet wines are, in most cases, intended for dessert. The high content of sugar makes this type of wine a suitable drink for the end of every meal as it can stimulate digestion and quench the appetite. In addition, some sweet wines can be served as appetizers.

Sweet wines require a greater addition of SO_2 not only for conservation, but also for stopping the fermentation to preserve some of the natural sugars in the wine. Over time, many researchers have focused on reducing the amount of SO_2 in wine by partially or totally replacing it with various oenological materials such as: lysozyme, potassium sorbate, dimethyl dicarbonate and, more recently, medium chain fatty acids (Antoce et al., 2005; Babikova et al., 2012, Santos et al., 2012; Baron et al. 2017).

Medium chain fatty acid derivatives (MCFAs, containing 6-14 carbon atoms) are natural compounds found in high concentrations in some foods and are a source of energy for the body (Michelle et al., 2019). Due to their ubiquity, they are used in various applications such as for the production of cosmetics, lubricants, biodiesel products or cleaning products.

Yeasts can also produce some MCFA and some of them have proven anti-fungal properties (Guilloux-Benatier et al., 1998; Antoce et al., 1997, 1998). However, few studies are available in the literature (Froissard et al., 2015).

Octanoic acid (C8) and decanoic acid (C10) belong to the group of Medium Chain Fatty Acids (MCFA). They are naturally present in wine, even though in a low concentration, being produced by yeasts during alcoholic fermentation as by-products of lipid synthesis (Legras et al., 2010). In oenology, they are studied as inhibitors of alcoholic and malolactic fermentation (Baron et al., 2011; 2014). During the fermentation of the must, the toxicity of these fatty acids for the microorganisms is increased by ethanol and low pH, which favours their penetration into the cells by passive diffusion through membranes in a non-

ionized form, dissociating afterwards at higher internal pH, leading to a decrease of the intracellular pH and disturbing homeostasis (Antoce et al., 1998; Legras et al., 2010).
Beside their antimicrobial activity, MCFA, together with their esters formed during alcoholic fermentation, can have a significant flavour effect on wine due to their higher solubility and volatility (Waterhouse et al., 2016).
For this study, 'Tămăioasă românească' variety was selected as a model for the production of sweet white wines. This aromatic muscat-type grape variety is very suitable for the production of natural sweet wines, due to its high sugar accumulation.
In order to obtain sweet wines by preserving some of the natural sugar from grapes, often the fermentation should be stopped. Usually, the fermentation is stopped by adding high doses of SO_2 and lowering the temperature in the fermentation tank. In an attempt to reduce the effects of high doses of SO_2 which may be less tolerated by some people, but still obtain a similar effect, in this study we used various doses of MCFA and lower doses of SO_2 to halt the alcoholic fermentation.

MATERIALS AND METHODS

'Tămăioasă românească' musts were produced from grapes harvested at 230 g/l sugar, crushed, and macerated for 8 hours at 14-15ºC.
Two winemaking protocols were used, the main difference among them being the yeast inoculated for the fermentation: ERSA (*Saccharomyces cerevisiae* provided by Enologica Vason) and TR313 (*Saccharomyces cerevisiae bayanus* from Rennaisance Yeast), respectively.
The addition of the MCFA for the inhibition of alcoholic fermentation was performed in the must, when the fermentation was med-way and the sugars reached about 10% Brix (more precisely, 10.0% Brix for the musts with TR313 yeast and 10.8% Brix for musts with ERSA).
The reagents used were as follows: octanoic acid (n-caprylic acid) from Kishida Chemicals, Osaka, Japan, purity >98.0%, decanoic acid (n-capric acid) from Wako Pure Chemical Industries, Osaka, Japan of purity >98% and sulphur dioxide solution of 6% from Miflachim Group, Romania.
The doses used for inhibition of alcoholic fermentation are summarised in Table 1, for each yeast experimental variant resulting 9 samples and 3 repetitions each.
The sugar consumption during fermentation was closely followed in each batch and at around 10% Brix the fermenting musts were treated with the specified doses (Table 1) of SO_2, octanoic acid and decanoic acid.
After the wines were clarified with 0.6 g/l bentonite and racked, all the samples were analysed in laboratory. The main wine parameters were determined in accordance with the OIV methods: alcoholic concentration was determined by distillation method OIV-MA-AS312-01A, sugar concentration in must by refractometric method OIV-MA-AS2-02 and in wine by the chemical method OIV-MA-AS311-01A, total acidity and pH by potentiometric method OIV-MA-AS313-01 (OIV, 2019).

Table 1. Wine samples coding and the doses of the inhibitors used for halting the alcoholic fermentation

No.	Repetitions	Samples coding*		Octanoic acid mg/l	Decanoic acid mg/l	SO_2 mg/l
		Yeast TR313	Yeast ERSA			
1-3	3	TR_313_0	TR_ERS_0	-	-	120
4-6	3	TR_313_OC10	TR_ERS_OC10	10	-	60
7-9	3	TR_313_OC20	TR_ERS_OC20	20	-	60
10-12	3	TR_313_OC30	TR_ERS_OC30	30	-	60
13-15	3	TR_313_DE10	TR_ERS_DE10	-	10	60
16-18	3	TR_313_DE20_r1	TR_ERS_DE20	-	20	60
19-21	3	TR_313_DE30_r1	TR_ERS_DE30	-	30	60
22-24	3	TR_313_OC10DE10	TR_ERS_OC10DE10	10	10	60
25-27	3	TR_313_OC15DE15	TR_ERS_OC15DE15	15	15	60

*TR= 'Tămăioasă românească' variety; 313 is abbreviation for the yeast TR313 used for fermentation (not to be confused with the variety name only 313 from the yeast name was used); ERS is abbreviation for the yeast ERSA. Also, octanoic and decanoic acids were abbreviated as OC and DE.

RESULTS AND DISCUSSIONS

For the wine samples prepared with several combinations of MCFA the influence of some major wine parameters was determined and discussed.

Influence of MCFA on pH

The management of pH in winemaking is of importance because this parameter impacts the colour stability, the precipitation of tartaric salts, the preservation of aroma compounds of the wine, being involved in the chemical reactions that are related to the formation, degradation and loss of several compounds in the wine. At the same time, the pH can influence directly or indirectly the protection against microorganisms that can alter the wine.

pH is the main parameter which determines the antimicrobial activity of the sulphur dioxide. The lower the pH, the higher the activity of sulphur dioxide used, because the same dose of free SO_2, at lower pH, has a higher ratio of molecular SO_2, which is the active form against microorganisms (Viegas et al., 1989).

Because MCFA are organic acids, they may also have influences on the pH of the final wines. Also, their inhibitory action is correlated to their molecular form (Antoce et al., 1997), which interferes at the membrane level of the microorganisms, disturbing the absorption of nutrients (Stevens and Hofmeyr, 1993).

The influence of the used doses of MCFA are presented, for both yeast fermentations, in Figure 1.

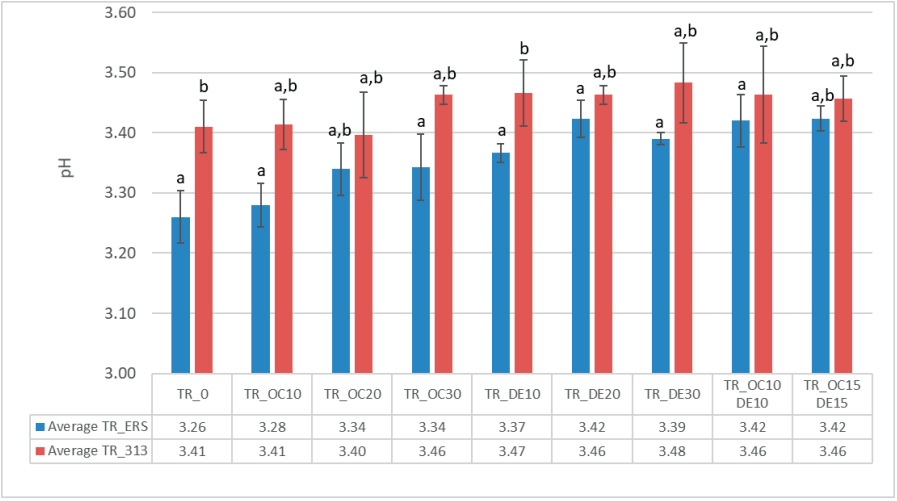


Figure 1. Mean ± SE of the pH of 'Tămăioasă românească' wine samples in which octanoic (OC) and decanoic (DE) acids in doses of 10, 15, 20 and 30 mg/l were used to stop alcoholic fermentation. Blue bars represent wines fermented with ERSA yeast and the red bars represent wines fermented with TR_313 yeast. Different letters represent significant differences of the means at the level of $p \leq 0.05$ in accordance with the post-hoc Tukey's Test run for 2-way-ANOVA analysis

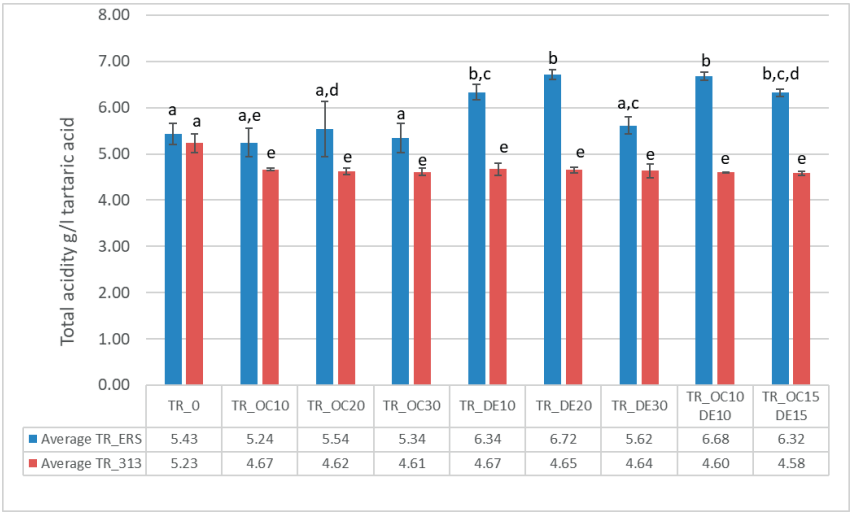
At the MCFA doses employed, only small pH differences are determined, with no statistical differences among samples fermented with the same yeasts. However, the yeast used for fermentation induced statistically significant differences in the final wine pH, from 3.3 to 3.4 for ERSA and 3.4 to 3.5 for TR_313. It is clear that these differences were induced due to different rates of fermentation and metabolites produced by the specific yeasts and not by the doses of MCFA used, as the control wine pHs are, for the same yeast, in the same range as the samples treated with fermentation inhibitors.

Irrespective of the yeast, a small tendency to increase the pH is observed in the samples treated with MCFAs, but the fermentation medium had sufficiently high buffering power to prevent the pH increase. Thus, irrespective of the octanoic or decanoic acid dose used to inhibit fermentation, the pH of the final wine did not rise sufficiently to be statistically significant.

However, this tendency of the acids to impact the medium pH, even in a very discrete, statistically not significant way, may have a slight contribution to the inhibition power of these acids, as well as of the SO_2, for all these inhibitors lower doses being necessary to have the same antimicrobial effect at a lower pH than at higher ones.

Influence of MCFA on total titratable acidity

Closely related to the pH, which is the real acidity perceived, the total titratable acidity is a parameter with more complex influence on the product stability and sensory characteristics. The amount of the typical acids in must and wine can vary within fairly wide limits, depending on the climatic conditions of the year, the degree of ripeness of the grapes, soil type, phytosanitary status of grapes, the processing of grapes, the storage of wine and more. Beside the influence on the pH, stability of salts, phenols and colour, chemically, these acids can interact with alcohols and form esters, some contributing to wine aroma as well. As also stated before, many acids directly influence the growth of microorganisms, being beneficial for the final product stability.



	TR_0	TR_OC10	TR_OC20	TR_OC30	TR_DE10	TR_DE20	TR_DE30	TR_OC10 DE10	TR_OC15 DE15
Average TR_ERS	5.43	5.24	5.54	5.34	6.34	6.72	5.62	6.68	6.32
Average TR_313	5.23	4.67	4.62	4.61	4.67	4.65	4.64	4.60	4.58

Figure 2. Mean ± SE of the total titratable acidity (expressed in g/l tartaric acid) of 'Tămăioasă românească' wine samples in which octanoic (OC) and decanoic (DE) acids in doses of 10, 15, 20 and 30 mg/l were used to stop alcoholic fermentation. Blue bars represent wines fermented with ERSA yeast and the red bars represent wines fermented with TR_313 yeast. Different letters represent significant differences of the means at the level of $p \leq 0.05$ in accordance with the post-hoc Tukey's Test run for 2-way-ANOVA analysis

Thus, the acidity of the wine can directly or indirectly influence the inhibition of alcoholic fermentation and act synergistically with the added MCFA and SO_2.

In correlation with the observation on the overall wine pH, the total acidity (Figure 2) was higher in the samples with lower pH. This inverse correlation is especially very clear in most of the samples fermented with ERSA yeasts. The wines fermented with TR_313 yeasts and added with any of the octanoic or decanoic acid dose found their stability at lower levels as compared with the control fermented in the absence of MCFA (5.2 g/l total acidity). This effect was correlated with the tartaric acid salts precipitation in these wines and led to a quite similar final acidity, at around 4.6 g/l.

In the wines fermented with ERSA yeasts we observed that the addition of the MCFA led to increases in total acidity (5.5-6.7 g/l total acidity in tartaric acid units) as compared to the control wine fermented with the same yeast (5.4 g/l total acidity), especially the wines added with decanoic acid alone or in combination with octanoic acid having statistically higher acidities as compared with control. This observation may indicate that the inhibitory action of decanoic acid may be more

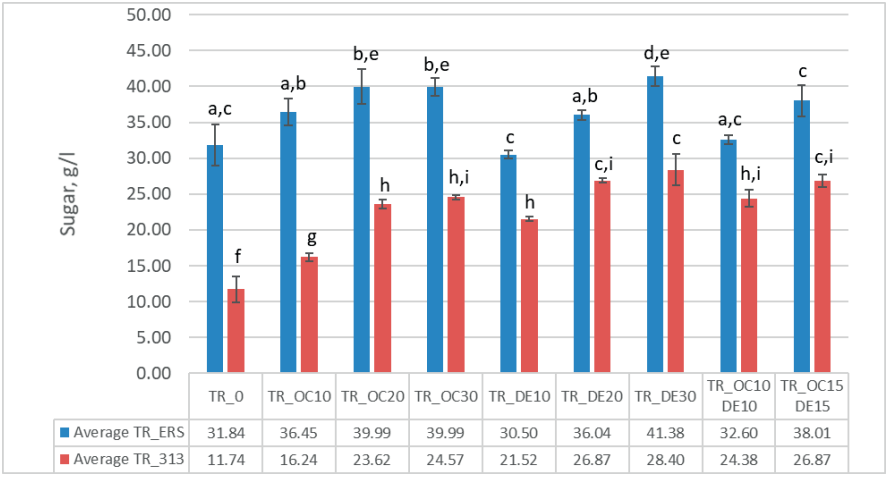
important than the one exerted by octanoic acid. Thus, it is to be expected that decanoic acid is better at inhibiting the alcoholic fermentation than its inferior homologues in the saturated fatty acid series.

Influence of MCFA on residual sugar content

The residual sugar content, that is the sugar concentration naturally remaining in wine after the cessation of fermentation, is a parameter mostly determined for the classification of wine from a legislative point of view in dry (< 4 g/l sugars as glucose and fructose), half-dry (4.01-12 g/l sugar), half-sweet (12.01-45 g/l sugar) and sweet (>45 g/l sugar). At the same time sugar plays an overwhelming role in the sugar-acidity balance of wines and in the general sensory perception.

As a consequence, sweet wines are preferred for certain food association and consumption patterns, being increasingly popular in the world as niche products, suitable for fast consumption, but also for long aging. For this reason, this study focuses on finding an alternative method to stop the alcoholic fermentation and leave a certain amount of unfermented sugar in the final wine.

The sugar content of the must in fermentation is often correlated with the existing population of active yeasts and, obviously, inversely correlated to the alcohol formed from it by the yeasts. Choosing the optimum moment for the inhibition of alcoholic fermentation is not an easy task, as it depends very much, beside the composition of the must, on the fermentation rate of the population of active yeasts. The higher or stronger the population of active yeasts, the higher the doses of inhibitors required to stop the fermentation.



	TR_0	TR_OC10	TR_OC20	TR_OC30	TR_DE10	TR_DE20	TR_DE30	TR_OC10 DE10	TR_OC15 DE15
Average TR_ERS	31.84	36.45	39.99	39.99	30.50	36.04	41.38	32.60	38.01
Average TR_313	11.74	16.24	23.62	24.57	21.52	26.87	28.40	24.38	26.87

Figure 3. Mean ± SE of the sugar content of 'Tămăioasă românească' wine samples in which octanoic (OC) and decanoic (DE) acids in doses of 10, 15, 20 and 30 mg/l were used to stop alcoholic fermentation. Blue bars represent wines fermented with ERSA yeast and the red bars represent wines fermented with TR_313 yeast. Different letters represent significant differences of the means at the level of $p \leq 0.05$ in accordance with the post-hoc Tukey's Test run for 2-way-ANOVA analysis

The inhibition should be started when the sugar concentration is 10-20 g/l higher than the final desired content, to allow for the inhibition process inertia. Higher doses of MCFA, coupled with sulphur dioxide addition, are proven to be more efficient in rapid inhibition, which correlates with higher residual sugar content in the final wine (Figure 3).

Even though the moment of inhibition was similar for all the wine samples, the highest sugar concentrations remained in the wines fermented with ERSA yeast (between 32-41 g/l sugar), as opposed to wines fermented with TR-313 yeast (between 12-28 g/l sugar). For either yeast, the control wine, inhibited only with SO_2 (120 mg/l), showed the lowest

residual sugar content of the series, in the same range of concentrations specific for the wines fermented with that yeast, but also inhibited by the addition of 10-30 mg/l doses of MCFA plus a half dose of SO_2 (60 mg/l). Accordingly, these results can be correlated with specific yeast behaviour, showing that TR-313 yeast, a *Saccharomyces cerevisiae bayanus* yeast, is more resistant to inhibitors and a faster fermenter than ERSA yeast, a classical *Saccharomyces cerevisiae* yeast. Thus, the cessation of fermentation needs to be started earlier in case of stronger yeasts such as TR-313, to accommodate for the longer inhibition inertia and to obtain wines with higher concentration of sugar, as it happens for more sensitive yeasts, such as ERSA. The inhibition inertia observed for the two yeasts can be explained mostly on their different resistance to sulphur dioxide, rather than to MCFAs. This fact is clear for the behaviour of the control wines, which were only added with sulphur dioxide as an inhibitor, at the same stage of their fermentation, but led to a quicker cessation of alcoholic fermentation in the case of ERSA (31.8 g/l sugar remaining) and a slower cessation in the case of TR-313 (11.7 g/l sugar).

It is worth noticing that the inhibition of fermentation tends to be dose-dependent. The effect was more evident in the case of ERSA, but also sufficiently evident in the case of TR-313 yeast.

As seen in Figure 3, in the range of 10-20 mg/l, the higher the concentration of the acid, the lower the sugar content was in the final wine. The doses of 30 mg/l have also a tendency to exert a higher effect, but in most cases the result obtained with 30 mg/l octanoic or decanoic acid was not significantly different than in the case of 20 mg/l of the same acid and same fermentation yeast. Only for ERSA 30 mg/l decanoic acid produced higher residual sugar in the final wine (41 g/l) as compared to 20 mg/l decanoic acid (36 g/l).

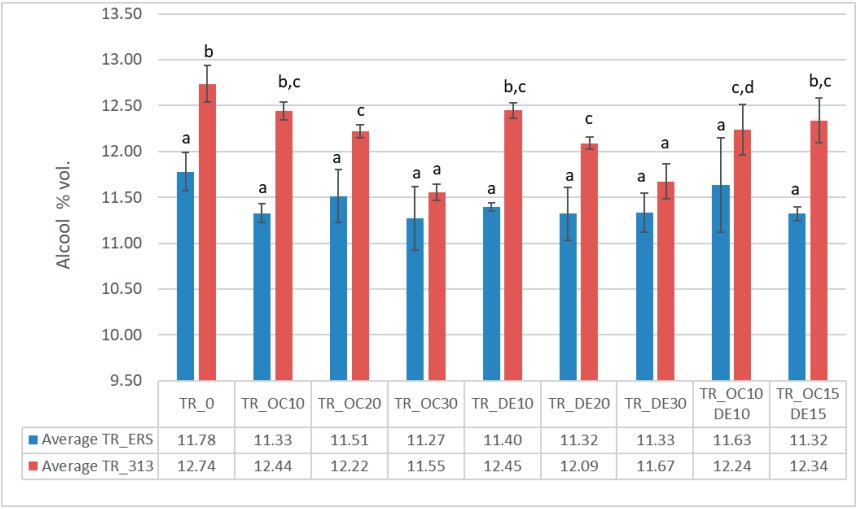
Although it was expected to observe a clearer higher effect with the decanoic acid, which has a higher molecular weight, our experiment did not lead to a conclusive result. Doses of 30 mg/l decanoic acid were more inhibitory than 30 mg/l of octanoic acid in both type of wines, irrespective of the fermentation yeast the results being a higher concentration of residual sugar when inhibition occurred in the presence of decanoic acids. For doses lower than 30 mg/l the results are mixed and more research is needed to confirm that decanoic is indeed more inhibitory for alcoholic fermentation in wine. The results produced by mixtures of octanoic and decanoic acids fall closely to the results obtained with single acids of similar doses, for the same fermentation yeast.

At the same time, the effect of these acids is amplified in samples that have a much lower pH and a much higher alcohol concentration, such as TR-313.

Influence of MCFA on alcohol content

Following the inhibition of alcoholic fermentation at a certain point to obtain wine with a desired residual sugar, alcohol concentration produced is lowered, in direct correlation with the sugar remained unconsumed. The alcohol concentration however is the main wine parameter, with legal and sensory importance. Moreover, even the inhibition of fermentation based on the addition of MCFAs is dependent on the alcohol concentration, their effect against yeasts being potentiated by each other. The higher the concentration of alcohol in the medium, the better is the inhibitory effect on microorganisms exerted by the addition of MCFA and/or SO_2. Thus, for a faster effect, the addition of octanoic and decanoic acid should be done when alcohol is already produced in sufficient quantities.

In the final wines, clearly correlated with the sugar concentrations remained unfermented, the alcohol concentration is lower in the sweeter wines. Accordingly, as explained in the previous section, the wines produced with the yeast more resistant to sulphur dioxide inhibition, TR-313, were able to ferment longer, even in adverse conditions, therefore contained in the end lower sugar concentrations (Figure 3), but higher concentrations of ethanol (Figure 4-red bars).



	TR_0	TR_OC10	TR_OC20	TR_OC30	TR_DE10	TR_DE20	TR_DE30	TR_OC10 DE10	TR_OC15 DE15
Average TR_ERS	11.78	11.33	11.51	11.27	11.40	11.32	11.33	11.63	11.32
Average TR_313	12.74	12.44	12.22	11.55	12.45	12.09	11.67	12.24	12.34

Figure 4. Mean ± SE of the alcohol content of 'Tămăioasă românească' wine samples in which octanoic (OC) and decanoic (DE) acids in doses of 10, 15, 20 and 30 mg/l were used to stop alcoholic fermentation. Blue bars represent wines fermented with ERSA yeast and the red bars represent wines fermented with TR_313 yeast. Different letters represent significant differences of the means at the level of $p \leq 0.05$ in accordance with the post-hoc Tukey's Test run for 2-way-ANOVA analysis

The less resistant yeast, ERSA (Figure 4-blue bars) is easily inhibited by both 120 mg/l SO_2 (control wine) and 10-30 mg/l doses of MCFA plus 60 mg/l SO_2, fermentation being stopped relatively at the same alcohol concentration (11.3-11.8% vol./vol. ethanol). No statistical difference was observed for the mean alcoholic fermentation of ERSA yeast samples, irrespective of the inhibitors or their doses.

In the case of the resistant yeast, TR-313, the values recorded for alcohol content in the final wines were significantly different (11.6-12.7% vol./vol. ethanol). The lower doses of MCFA (10 mg/l octanoic, 10 mg/l decanoic acid and some mixtures) did not lead to alcoholic concentrations significantly different than the control (range 12.4-12.7% vol./vol. ethanol). However, starting with 20 mg/l MCFA doses, the mean concentrations became significantly different than the control, the higher the dose, the less alcohol determined in the final wine. Also, the doses of 30 mg/l produced stronger significant effects than 20 mg/l doses, irrespective of the fatty acid used. The effect of the combinations of octanoic and decanoic acids however, did not display an additive effect, but rather behaved as the single 10 mg/l doses of MCFA. More research is needed to confirm or not this effect and to understand the potential mechanism.

As control samples for both yeasts contained the highest alcohol concentrations of their wine series, it is clear that sulphur dioxide alone, even in dose of 120 mg SO_2, was not as effective as the MCFA addition combined with a lower SO_2 dose of 60 mg/l.

CONCLUSIONS

The results performed on the 'Tămăioasă românească' variety highlight the efficiency of MCFA as antifungal agents able to inhibit alcoholic fermentation and leave some of the natural sugar unfermented. MCFA addition is thus a good alternative method to produce sweet of half-sweet wines.

The study showed that to halt the alcoholic fermentation the dose of medium chain fatty acid used is mainly correlated to the inhibitory effect, but also the type of acid (octanoic or decanoic in our case) can have influences, especially due to their potentially different effect on total acidity and pH. With a higher size and more clear influence on acidity, decanoic acid was expected to be more inhibitory than octanoic, but in the case of final sugar content in wines this assumption proved to be correct only for the higher doses (30 mg/l). Doses of singular acids of 10 mg/l are effective, but 20 mg/l are better, while 30 mg/l

seem to lead in many cases to similar effects as 20 mg/l. Also, the combinations of both acids do not prove to be better in any way than single acid, but more research may be needed in this respect.

Most importantly, the study showed that the yeasts used for wine fermentation react differently to the endeavours to stop the fermentation, their resistance to sulphur dioxide inhibition having a decisive role, as sulphur dioxide in lower doses is used to complement the action of MCFA. Irrespective of the yeast, however, by using MCFA the dose of sulphur dioxide required to stop alcoholic fermentation can be lowered.

To obtain best results, the process of fermentation cessation should be started earlier during fermentation for the yeasts more resistant to the action of sulphur dioxide, to compensate for the time required to overcome the inertia to the inhibition, during which the sugar is still consumed and transformed in ethanol.

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