THE USE OF SATURATED MIDDLE-CHAIN FATTY ACIDS IN THE TECHNOLOGY OF WINE PRODUCTION

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Abstract: Reducing the amount of sulphur dioxide in wine has been one of the main subject of interest to winemakers for several years. Recent research has shown the efficacy of saturated middle chain fatty acids (MCFAs) as inhibitors of alcoholic fermentation; even in the production of wine with higher residual sugar. In this study, the effectiveness of the octanoic (C_8), decanoic (C_{10}) and dodecanoic (C_{12}) mixture of MCFAs as a complement to sulphur dioxide was monitored with a view to reducing the dosage of sulphur dioxide required to ensure effective inhibition of *Saccharomyces cerevisiae* yeasts, which in turn halts alcoholic fermentation. The progression of alcoholic fermentation was observed across twenty-four variants and combinations of middle chain fatty acid mixtures and sulphur dioxide. The development and conclusion of alcoholic fermentation was controlled for ten days, after application of the MCFAs mixtures. The process was monitored through daily measurement of residual sugar content. The results were statistically evaluated. A mixture of the C_8, C_{10} and C_{12} MCFAs in the ratio 2:7:1, in concentration 10 mg/l, can reduce the required dosage of sulphur dioxide by several tens of mg/l. The results prove that the application of 10 mg/l of MCFAs mixture with 30–40 mg/l of sulphur dioxide has the same efficacy as the dosage of 60 mg/l of sulphur dioxide alone.

Key Words: alcoholic fermentation, octanoic acid, decanoic acid, dodecanoic acid, sulphur dioxide

INTRODUCTION

One of the main subject of interest in the wine world in recent years has been the reduction of sulphur dioxide in wine. This is due to the possible health risks associated with the consumption of SO_2. Whilst the total exclusion of sulphur dioxide without any substitution is, in most cases, impossible, the dosage of SO_2 could be significantly reduced. Sulphur dioxide has many important functions in the winemaking process – it acts as an antioxidant, deactivates enzymes and has antimicrobial effects. Furthermore, SO_2 affects the organoleptic characteristics of wine, both positively and negatively (Baroň 2013, Guerrero and Cantos-Villar 2015, Henderson 2009).

Whilst the health risks associated with the consumption of SO_2 are a serious cause for concern, the issues associated with using no SO_2 in the winemkaing process forced us to consider alternatives. Much attention had been focused on identifying alternatives or substitutes which were effective in protecting wine and minimising or eliminating the use of sulphur dioxide, without any negative influence on human health. However, to the extent that such chemical or physical alternatives had been identified, those alternatives were prohibitively expensive and/or technically demanding and therefore unavailable to most winemakers (Baroň 2013).

A breakthrough in this area has been made through the exploration of saturated MCFAs, which are perfect alternatives to sulphur dioxide. MCFAs can replace the SO_2 in respect of antimicrobial function. Fatty acids with longer chains (C_{16} and C_{18}) work like activators of alcoholic fermentation (Rodriguez-Nogales et al. 2013), whereas MCFAs (C_8, C_{10} and C_{12}) have the opposite effect, halting alcoholic fermentation. MCFAs are also good at inhibiting undesirable malolactic fermentation (Guilloux-Benatier et al. 1998, Viegas and Sá-Correia 1997). The functioning of MCFAs C_8, C_{10} and C_{12} is very straightforward. They penetrate the body of the yeast, changing the structure of the yeast resulting in the yeast cell becoming permeable to other substances. Thus, the yeast becomes non-functional and alcoholic fermentation is halted. These MCFAs are able to prevent refermentation which could otherwise occur in the wine bottle as a result of the presence of residual sugar. Accordingly, MCFAs could assist with the storage of wine. The question is, are there residues of the MCFAs in wine as a result of this process? This can be answered very easily. A significant proportion of the MCFAs added to the wine are absorbed or assimilated by the yeasts. The balance of MCFAs are esterified and remain in the wine as residue; however, this is a negligible quantity in the order of tenths (maximally units). MCFAs are fixed on the dead-yeast bodies and are removed gradually during the winemaking process. No significant difference has yet been found between residues of MCFAs and their esters in wine treated with or without MCFAs. Simultaneously, no increased occurrence of volatile compounds was registered. The aim of this study was to explore these new issues, which appeared in the wine sector. The research followed the idea of using MCFAs in winemaking technology and the storage of wine (Baroň 2013).

The main goal of this experiment was to confirm or disprove the efficacy of MCFAs – octanoic, decanoic and dodecanoic – as inhibitors and stoppers of alcoholic fermentation.

MATERIAL AND METHODS

Design of experiment

The progression of alcoholic fermentation was compared across twenty-four different variants and combinations of dosages of MCFAs mixtures and sulphur dioxide. The evolution of alcoholic fermentation and its conclusion was controlled for ten days, after application of MCFAs mixtures and SO_2. The term of stopping alcoholic fermentation was determined at the time, while the content of fermentable sugar in the fermenting must was 25.6 mg/l (in this case it is called “the day 0”). The process was monitored daily through the measurement of residual sugar content. The results were statistically evaluated.

Table 1 Analytical parameters of must at the moment of addition of MCFAs mixture and SO_2

PARAMETER	Titratable acids	pH	Alcohol content	Content of residual sugars
VALUE	9.43 g/l	3.12	11.15% vol.	25.6 g/l

Material

The must from the *‘Laurot’* (rosé) variety was chosen for this experiment.

Sulphur dioxide was used in solid form – as $K_2S_2O_5$ (also called pyrosulphate), which was quantitatively converted into a pure form (from a practical point of view).

The mixture of MCFAs: for this experiment a MCFAs mixture with the ratio $C_8 : C_{10} : C_{12}$ = 20 : 70 : 10 was chosen for every combination with or without sulphur dioxide (except the control variant, which was without any treatment). 100 mg of MCFAs (in the appropriate ratio) was dissolved in an aqueous solution of potassium hydroxide (volume 1L).

The variants and combinations of dosages of SO_2 and MCFAs are shown in Table 2 below.

Table 2 Variants and combinations of dosages of MCFAs and SO_2

MCFAs/SO_2 [mg/l]	0	20	30	40	50	60
0	0/0	0/20	0/30	0/40	0/50	0/60
5	5/0	5/20	5/30	5/40	5/50	5/60
10	10/0	10/20	10/30	10/40	10/50	10/60
20	20/0	20/20	20/30	20/40	20/50	20/60

Methods

Determination of analytical parameters of must was completed after the application of the MCFAs mixture and SO_2, and was measured by the ALPHA analyser, which is working on principle infrared spectroscopy with Fourier’s transformations (FTIR) using the sampling technique Attenuated Total Reflection (ATR). Basic analytical parameters were established on this analyser; these parameters were: titratable acids, pH, alcohol content and residual (fermentable) sugar content (Table 1).

The residual sugar content after treatment (application of MCFAs and SO_2) was measured with Rebelein's method. The principle of this method consists of iodometric determination based on the difference in consumption of sodium thiosulphate on titration of a defined concentration of the copper cation and its balance after reaction with reducing sugars of wine without removal of interfering substances (Balík 1998).

RESULTS

This study examines the identification of a substance to complement sulphur dioxide as a wine additive, which could mean a significant reduction of sulphur dioxide dosages. One of the very problematic aspects of making wine with a higher content of residual sugar is stopping alcoholic fermentation. This step usually needs a higher dose of SO_2. The reduction of this dose could be supported by the application of a MCFAs mixture (C_8, C_{10} and C_{12}). This MCFAs mixture is able to stop alcoholic fermentation, so the dose (and thus the content) of sulphur dioxide could be greatly decreased.

The most important parameter monitored in this experiment was the change in residual sugars content. By observing this change, the effectiveness of the MCFAs mixture in stopping alcoholic fermentation could been determined. The efficacy of the MCFAs mixture (octanoic, decanoic and dodecanoic acid) is summarized and commented upon in the figures below.

Table 3 Key to the charts

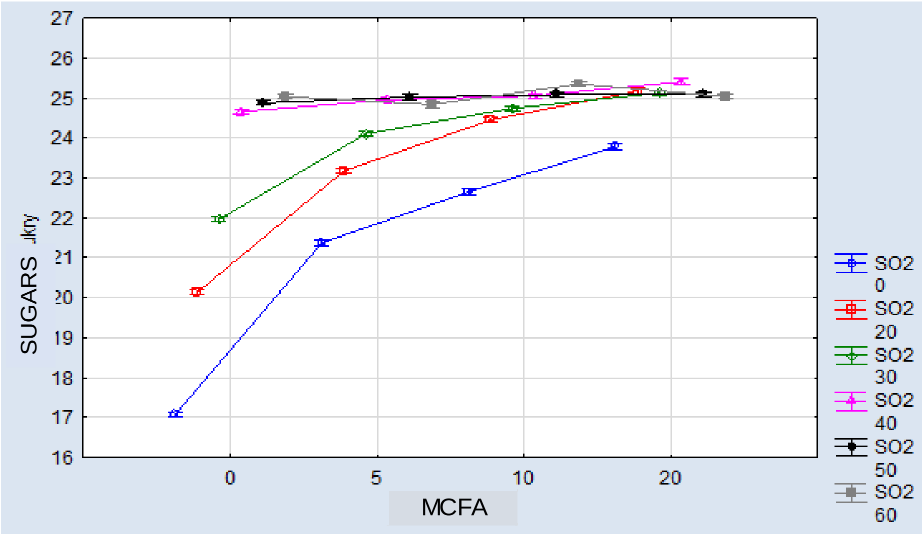
ABBREVIATION	EXPLANATORY NOTE	ABBREVIATION	EXPLANATORY NOTE
MCFA 0	Without addition of MCFAs	SO_2 0	Without addition of SO_2
MCFA 5	Addition of 5 mg/l MCFAs	SO_2 20	Addition of 20 mg/l SO_2
MCFA 10	Addition of 10 mg/l MCFAs	SO_2 30	Addition of 30 mg/l SO_2
MCFA 20	Addition of 20 mg/l MCFAs	SO_2 40	Addition of 40 mg/l SO_2
Sugars	Content of residual sugars [g/l]	SO_2 50	Addition of 50 mg/l SO_2
Days	Measurement 20. – 29. 11. 2015	SO_2 60	Addition of 60 mg/l SO_2

The efficacy of sulphur dioxide depending on the different doses of MCFAs mixture

MCFAs were investigated for the purpose of reducing the dosage (and the content) of sulphur dioxide in wine; not necessarily the total elimination of SO_2. Through analysis of the content of residual sugar after the addition of the MCFAs mixture and SO_2, it was possible to compare the efficiency of MCFAs and SO_2 during the halting of alcoholic fermentation. Through testing different variants and combinations of dosages of MCFAs mixture and SO_2, it was possible to find the appropriate solution for reducing the amount of sulphur dioxide required to be added. The following chart (Figure 1) shows the efficacy of sulphur dioxide in combination with MCFAs and the mutual influence of these substances. It can be said that the higher the content of fermentable sugars, the higher the effectiveness of the inhibitors. The SO_2 dose could be up to several dozen of mg/l lower in a suitable combination with the MCFAs, which confirms the idea that MCFAs enhance the efficiency of SO_2. The experiment was designed to consider the parameters influencing the development of fermentable sugar content - time, sulphur dioxide dose and medium chain fatty acid mixture.

The obtained results are shown at Figure 1 and from that follows the conclusion that the efficacy of sulphur dioxide in dosages more than 40 mg/l SO_2 (colours pink, black and grey) was almost identical in these variants. These dosages of SO_2 were able to stop alcoholic fermentation without the addition of the MCFAs mixture. What is more interesting is that the dosages of SO_2 in concentration 20 and 30 mg/l in combination with MCFAs mixture in concentrations 10 and 20 mg/l seem very promising, insofar as the inhibition of alcoholic fermentation is more than noticeable. The combination of a convenient dose of MCFAs mixture and a reduced dose of SO_2 (less than 40 mg/l) is more effective than a dose of the SO_2 by itself, even in the case of a higher concentration of SO_2. This supports the conclusion that a reduction in the dosage of sulphur dioxide is possible using the MCFAs mixture.

Figure 1 The degree of influence of SO_2 on the inhibition of the yeasts according to doses of MCFAs mixture is shown by the development of content of residual sugars (g/l)

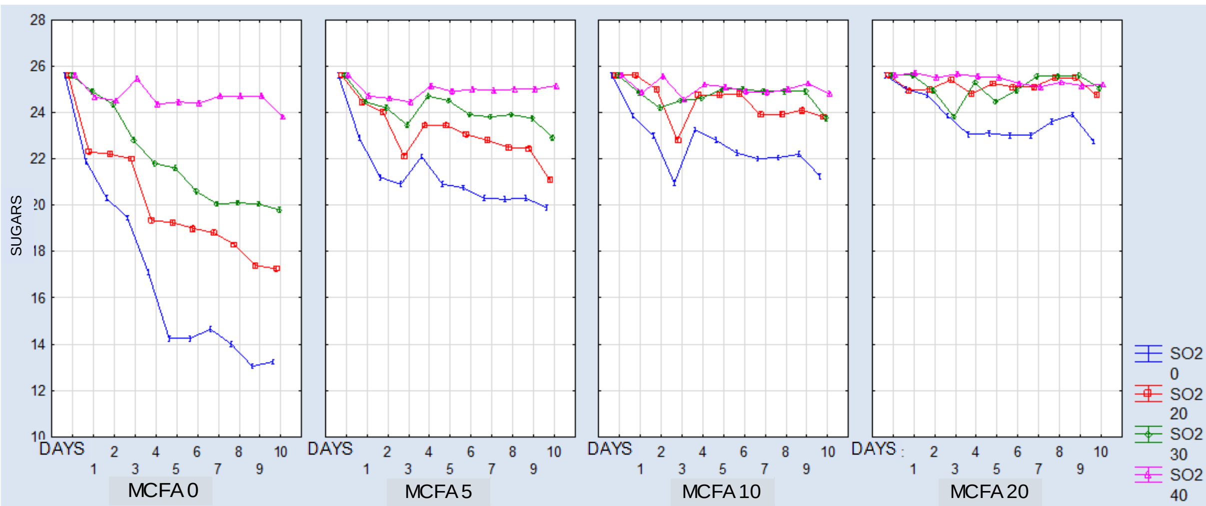


The development of the content of residual sugar depending on all monitored parameters

Figure 2 (below) provides the most comprehensive view of the entire experiment. The development of the content of residual sugars for all samples with the addition of sulphur dioxide 0–40 mg/l (included) is shown. The samples with doses of 50 and 60 mg/l were not considered in this evaluation, because those doses of SO_2 can stop alcoholic fermentation without the addition of the MCFAs mixture (Figure 1).

The experiment was designed to consider the parameters influencing the development of fermentable sugar content: time, sulphur dioxide dose and dose of MCFAs mixture. Applying those parameters, it was possible to find the most effective combination of the inhibitors (i.e. the MCFAs and SO_2) and select the best variant for stopping alcoholic fermentation, with the aim of making wine with a higher residual sugar content.

Figure 2 The development of residual sugar content depending on the added amount of MCFAs mixture, SO_2 and on time



The most significant results for the intended purpose are contained in the box labelled "MCFA 10" (Figure 2); this is the dose 10 mg/l of MCFAs mixture in combination with 20–40 mg/l of SO_2 (colours red, green and pink). This concentration is optimal for wineries, even if the goal of production is a wine with a higher residual sugar content. The change of residual sugars was, in this case, minimal. A little change can be attributed to the phasing of alcoholic fermentation when the measure was made, or potential measurement errors. Slowing of alcoholic fermentation can be observed from the dose 5 mg/l of MCFAs mixture even with zero addition of sulphur dioxide. Obviously, the variants with the highest addition of MCFAs mixture and SO_2 were the most effective (the box "MCFA 20"). There are only minimal differences in effectiveness between the different SO_2 dosages applied in this variant (units percent). The one outlier is where the MCFAs mixture was applied by itself, without SO_2. Also in this case we can see obvious slowing of alcoholic fermentation.

Table 4 Statistical evaluation – Fisher's test LSD

CONTENT OF SUGAR	AVERAGE MCFA	A	B
20	0.000000	****	
23	2.500000	****	****
24	7.500000	****	****
25	8.928571		****

The evaluation in Table 4 shows that the MCFAs averages (with the dose 30–40 mg/l SO_2) at similarity level $\alpha = 0.05$ were divided into three homogeneous groups. The MCFAs dose 0 mg/l is group A, the doses of MCFAs 2.5 and 7.5 mg/l are the group AB and the dose of MCFAs 8.93 mg/l (rounded) is the group B. The difference between the doses 0 and 8.93 mg/l of MCFA is confirmed by this test.

DISCUSSION

The MCFAs C_8, C_{10} a C_{12} were investigated many years ago (Garbay et al. 1995, Guilloux-Benatier et al. 1998, Viegas and Sá-Correia 1997, Cabral et al. 2001) and their ability to inhibit alcoholic fermentation and possibly halt undesirable malolactic fermentation was confirmed in several studies (Baroň 2013, Budínová 2016).

The structure of the experimental part of this study was relatively simple, but the results are conclusive. The efficacy of MCFAs was confirmed. A handicap of this experiment was a small spectrum of monitored factors that could affect the effectiveness of MCFAs.

The results confirm previous findings that a dose of MCFAs mixture for wine production of 10 mg/l is adequate to enable a reduction in the dose of sulphur dioxide by several tens of milligrams per litre (Baroň 2013). However, several questions remain. How high a dose would be required at a later stage (for example, during storage) to ensure microbial stability without refermentation. This problem relates to the possible development of resistance of the yeasts to MCFAs, because yeast cells can build their own defence mechanism. According to Cabral (2001), yeasts that have been exposed to the sub-lethal effects of octanoic acid at their inception are more resistant to the inhibitory effect of medium chain fatty acids. Further, he presents a very interesting finding about so-called "cross-resistance"; if a cell is infected by MCFAs and the permeability is increased, ethanol can flow into the body of the yeast and, whilst it could kill the yeast, the moderate stress induced by ethanol can also force the yeast to activate its own defence mechanism. Further, the yeast can use this increased permeability for faster transport of octanoic acid out of the cell. The issues of toxicity and time of effectiveness of the MCFAs require further clarification.

Budínová (2016) states in her thesis that the dose 20 mg/l MCFAs mixture without adding SO_2 was not sufficiently effective and, consequently, disadvantageous for the winemakers. This proposition is consistent with the results of our study. However, where the results differ is in the variant with 5 mg/l MCFAs and 60 mg/l SO_2. While this dose (5 mg/l) of MCFAs mixture (and also SO_2 in concentration 60 mg/l itself) was quite sufficient in our study, Budínová's research suggested that these doses were less effective than our study suggested. This difference could be explained by the content of residual sugar contained in the tested wines differing at the moment when the MCFAs and SO_2 were applied. In our experiment, the content of residual sugar was lower by 10–15 g/l than in Budínová's experiment.

It is very difficult to discuss results where there are only a few comparable studies, even where those studies resulted in similar conclusions.

CONCLUSION

This research was focused on identifying alternative substances to sulphur dioxide. Going back to the basics – SO_2 can stop alcoholic fermentation and ensure microbial stability of the wine. However, the risk of the yeast and the other microorganisms developing resistance is considerable, in addition to the possible health risks. It is no wonder that the reduction of SO_2 is one of the most discussed topics in the contemporary wine world. Our study proves that the amount of sulphur dioxide can be reduced by tens of milligrams thanks to the MCFAs mixture even if the aim is to produce a wine with a higher content of residual sugar. The ideal concentration of the dose of the MCFAs mixture is 10 mg/l. This dose and the method of application is acceptable, easy and inexpensive. The MCFAs mixture is a new method for the production of wine with a higher content of residual sugar. This method is not technologically difficult and therefore it is accessible to smaller winemakers. This method has minimum risks, because MCFAs are naturally occurring and fit for human consumption. The amount of MCFAs residue after the application of the MCFAs mixture as a treatment with the aim to inhibit yeasts differs only fractionally from wine which has not had the MCFAs mixture added. MCFAs cannot be detected in the organoleptic properties, if they are used sparingly. The middle chain fatty acids mixture seems like an ideal aid in the wine production process.

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