The use of saturated medium-chain fatty acids in wine production technology

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Abstract: The use of sulphur dioxide has a long history in viniculture. Due to its negative effect on human health, it is necessary to search for substances that could reduce, or even exclude, the need for sulphur dioxide in wine making. One option is use of saturated medium-chain fatty acids (MCFA) that can inhibit yeast activity. In this study, the effect of mixing octanoic acid (C_8), decanoic acid (C_{10}) and dodecanoic acid (C_{12}) in the ratio 2:7:1, respectively, on their residuals and sensory properties is examined. After application of six different concentrations in combination with two doses of SO_2, the MCFA content was measured and sensory analysis was performed. The results show that the higher the dose of MCFA is, the more of them were bound to the yeast bodies and the lower the content is in the wine. Sensory analysis shows a dose of 20 mg/l as a limit, below which consumers are unable to perceive differences among MCFA doses, and the odour of MCFA declines with the duration of wine ageing.

Key Words: saturated medium-chain fatty acids, octanoic acid, decanoic acid, sulphur dioxide, yeast

INTRODUCTION

Sulphur dioxide (SO_2) is a very important additive in wine production. It inhibits reproduction of yeasts, which causes deceleration or stopping of alcoholic fermentation. It also kills bacteria that can bring about the incidence of wine diseases. Concurrently, it deactivates oxidation enzymes and bonds oxidation products and also oxygen. Thanks to these effects, it has been used for many centuries. Although the addition of SO_2 to wine was temporarily prohibited in 15th century, its use, although still legally limited, has been preserved until today.

Most winemakers would agree that it is impossible to dispense with sulphur dioxide in wine production, it is only possible to limit its quantity. However, the fundamental problem remains that SO_2 is a major allergen (notification of its content at a concentration above 10 mg/l must be given) and consumption at higher doses causes health issues (headache, stomach ache, asthmatic problems) (Lester 2013); therefore, restriction of its application, due to the ever–increasing consumption of wine in the Czech Republic, is highly desirable.

This problem could be solved by the introduction of new substances (as a supplement to SO_2) that have the same effects on microorganisms as sulphur dioxide while not harming human health and not influencing the sensory properties of the wine. In this case, an interesting group is saturated medium-chain fatty acids. These compounds occur in wine naturally – they can be produced by yeasts themselves as intermediates of lipid synthesis during fermentation (Tehlivets et al. 2007). Some of them support alcoholic fermentation, such as palmitic acid (C_{16}) and stearic acid (C_{18}) but acids with shorter chains, octanoic acid (C_8), decanoic acid (C_{10}) and dodecanoic acid (C_{12}) cause its deceleration, particularly in late-fermentation phases (Viegas and Sa-Correia 1997). There are changes in the lipid build-up in the cytoplasmic membranes of yeast, the pH of the cell decreases and the proton gradient is disturbed (Alexandre et al. 1996), and cells then lose their viability.

The advantage of MCFA, unlike sulphur dioxide, is their harmlessness to health. They occur naturally in animal milk fats and coconut oil. However, their disadvantage is that they lack antioxidant effects. They can also influence the aromatic expression of the wine – their aroma resembles coconut, soap, dull fat, wax (Li et al. 2008). Due to low effective doses applied to wine, the risk is negligible.

The aim of this study is to monitor the effects of saturated medium-chain fatty acids used in wine technology, especially their behaviour after application to wine and their influence on the sensory properties of wine.

MATERIAL AND METHODS

Design of experiment

After the grapes were processed, the must was transferred to a stainless-steel tank, where alcoholic fermentation started after 48 hours. During the fermentation of the must to a residual sugar content of 17.6 g/l, 36 l of wine was taken and subsequently divided into 12 carboys with a volume of 3 l then further divided into two rows. Immediately after distribution, a mixture of saturated fatty acids was applied in quantities of 5, 10, 20, 30, 40 and 60 mg/l to each row. After 24 hours of application of the saturated fatty acids, a sample was taken from each carboy into a glass vial with a volume of 187 ml. All 12 samples were stored in the freezer. Subsequently, sulphur dioxide in the form of liquid sulphur (ammonium bisulphite) was applied at doses of 20 mg/l to one row and 40 mg/l to the second row. After 48 hours application of a mixture of saturated fatty acids, samples from each carboy were taken again. Sampling was again carried out after 168 hours and 672 hours (one month) of the application of sulphur dioxide.

Material

In this experiment the must of variety 'Hibernal' was used. Sulphur dioxide was used in liquid form as 40% ammonium hydrogen sulphite, namely Supersolfosol (BS Vinarske potreby, Czech Republic).

Saturated fatty acids were applied in the form of a mixture of octanoic acid (C_8), decanoic (C_{10}) and dodecanoic (C_{12}) with ratio 2:7:1, respectively. A hundred mg of the mixture was dissolved in 1 l of aqueous potassium hydroxide solution. This solution was applied to the wine in combination with sulphur dioxide, and these combinations are shown in the Table 1.

Table 1 MCFA and SO_2 application variants

Amount of SO_2 (mg/l)	Amount of MCFA (mg/l)					
20	5	10	20	30	40	60
40	5	10	20	30	40	60

Methods

In basic analysis of the must, the sugar content was determined refractometrically (Altago, Japan), titratable acid content and assimilable nitrogen content were determined using a TitroLine Easy automatic titrator (manufacturer SI Analytics GmbH, Germany) and the pH of the must was determined with a pH meter (WTW pH 526 and SenTix 21 pH electrode WTW, Germany). Residual sugar was determined with an ALPHA analyser (Bruker, USA) operating on the principle of Fourier transform infrared spectrometry using the Attenuated Total Reflection (ATR) sampling technique. To determine the content of saturated fatty acids before and after the mixture application, a gas chromatography mass spectrometer (GC-MS, Shimadzu, Japan) was used. The results were statistically processed (Statistics, StatSoft, USA).

RESULTS

Using the gas chromatograph, the content of the saturated fatty acid residues in the wine was analysed. Two variants of sulphur dioxide dosing, 20 mg/l and 40 mg/l, were used during the experiment. However, the results show that the sulphur dioxide content does not have a significant effect (α = 0.05) on the content of saturated MCFAs in the wine, and the residue is generally higher (not shown) at 40 mg/l; however, this difference is negligible. Also, the results of dodecanoic acid are not significant, therefore they are not shown.

MCFA content before the MCFA mixture application:

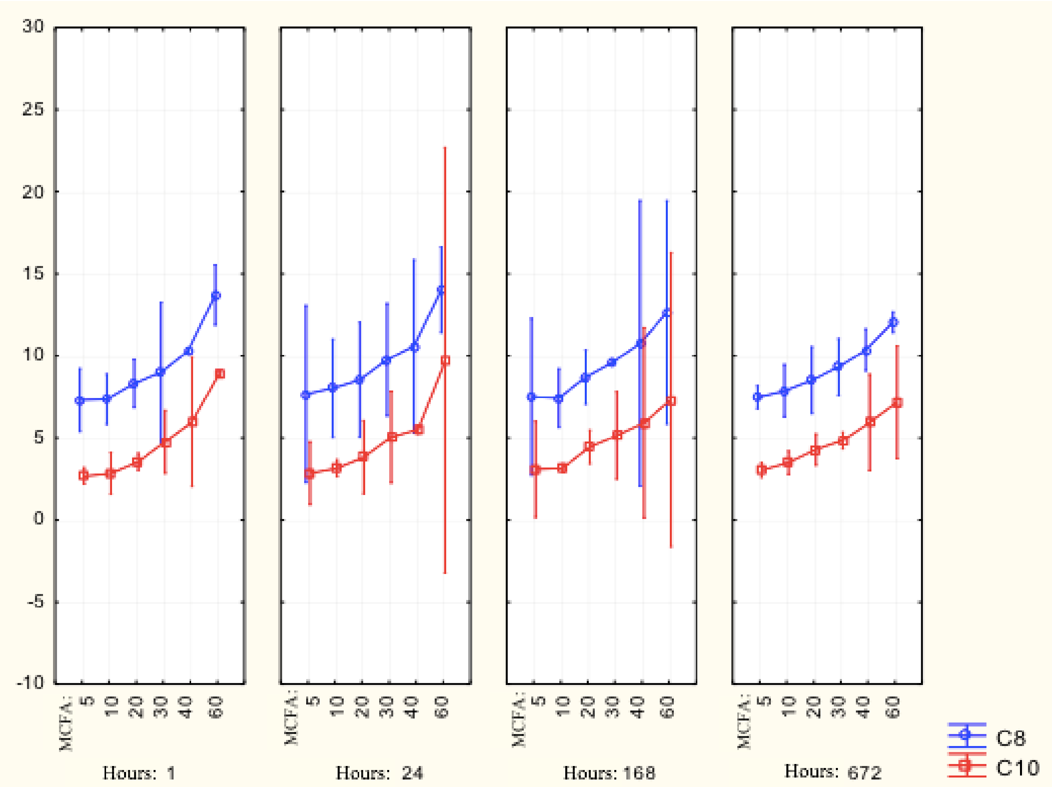
C_8 – 6.76 mg/l

C_{10} – 2.18 mg/l

MCFA content

Results of statistical analysis at a significance level of $\alpha = 0.05$ (Figure 1) show the differences between the individual resultant octanoic or decanoic acid values are not as significant as the differences between the original doses, while at a dose of 5 mg/l, approximately 40% of the MCFA content has been attached (final C_8 content 7.31–7.67 mg/l, C_{10} 2.68–3.09 mg/l), and more than 85% MCFA was attached at a dose of 60 mg/l (final C_8 content 12.05–14.025, C_{10} 7.17–9.72 mg/l). These results indicate an increasing ability of yeast to receive and bind saturated fatty acids with increasing dose of MCFA. Analyses were performed on samples 24 hours, 168 hours and 672 hours after SO_2 application. The resulting MCFA content remains almost unchanged over 672 hours.

Figure 1 Octanoic and decanoic acid content depending on MCFA dose



Sensory analysis

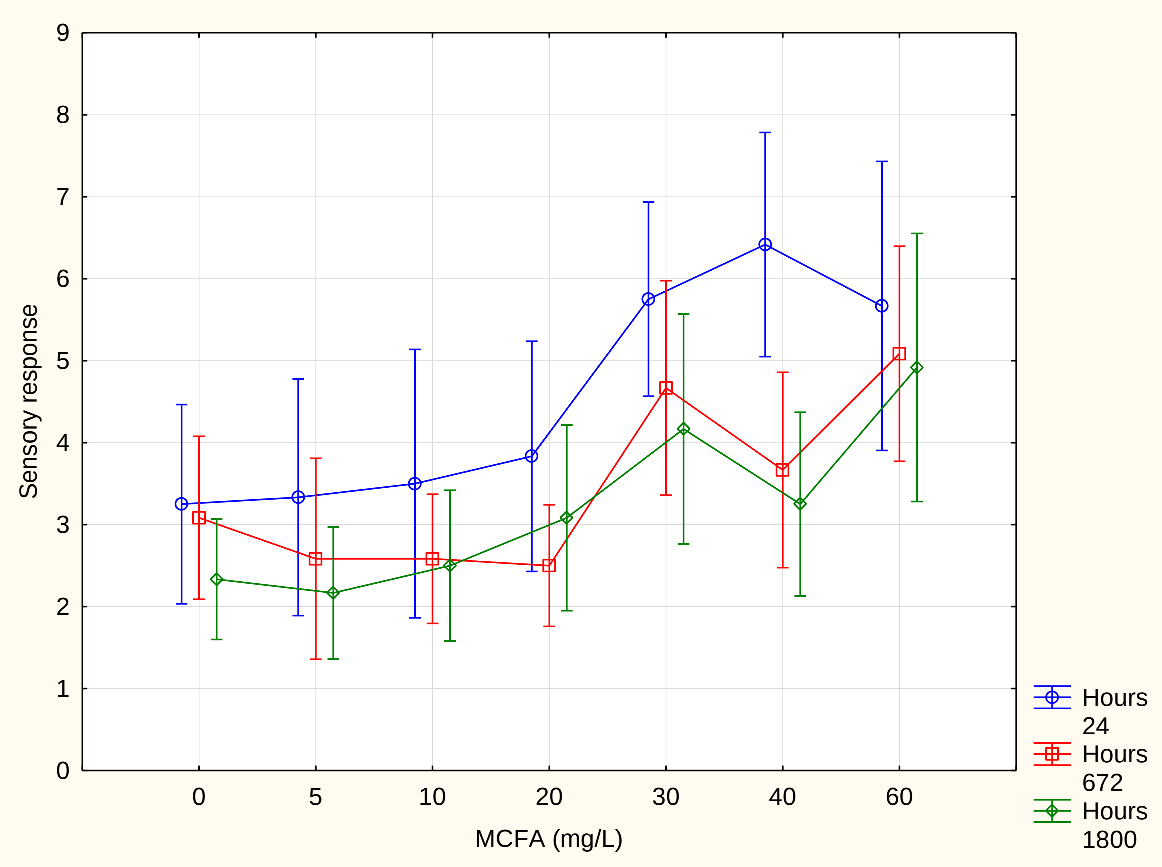
Sensory analysis of the wine was carried out 24 hours after SO_2 application, one month (672 hours) after SO_2 application and 3 months (1800 hours) after SO_2 application, for all 12 variants. Sensory expression of saturated MFCA, usually described as ‘coconut, soap, ductile fat, wax’, was evaluated. Each of twelve glasses contained one sample according to the variants listed in Table 1. The expression was scored points, ranging from 1 to10 points, with 10 being the best (the highest intensity of expression). The significance level of the statistical analysis was $\alpha = 0.05$.

An important limit in this study is 20 mg/l MCFA. At doses of 20 mg/l MCFA and less, the evaluator is not able to perceive differences between doses. At doses higher than 20 mg/l, the expression of MCFA increases and becomes more evident to the evaluator. However, at doses of 30, 40 and 60 mg/l the differences of perception are eliminated again, and the differences between the assigned points is minimal (Figure 2).

Figure 2 shows the MCFA sensory-expression evaluation. The most striking sensory response occurs 24 hours after the application of SO_2, and the lowest occurrence 1,800 hours after the application of SO_2. As the number of hours increases, the sensory effect of saturated fatty acids reduces. After application, the MCFAs are bound to the yeast bodies and the sludge particles are gradually deposited on the bottom of the vessel.

The amount of SO_2 applied does not have a pronounced effect on the expression of saturated middle-chain fatty acids.

Figure 2 Sensory response depending on time and dose of MCFA applied



DISCUSSION

It is important to detect substitutes or supplements of sulphur dioxide, due to their harmful effects on health. In several scientific papers (Baron 2013, Babikova et al. 2012), although still not quite enough the effect of MCFA added to wine on yeast during fermentation was investigated, although the effects of fatty acids have been a subject of research for a relatively long time (Viegas et al. 1989). The results of this study show that the more MCFA added, the more of them were received by the yeasts. Although the resulting residues were logically higher with higher dose added, the ratios of bound MCFA were different. This phenomenon could be explained by the fact that the more MCFA contained, the higher is the activity of cell carriers and the greater is the amount of MCFA the yeast receives. Given the ratio of MCFA in the mixture, there was a significantly higher intake of decanoic acid, which coincides with the results achieved by a team of French scientists (Lafon-Lafourcade et al. 1984). At the end of the fermentation due to increased membrane permeability, decreased ATP concentration and inhibition of ATPase may incur yeast-cell disruption and pouring out of their contents into the surroundings (Viegas et al. 1989).

The sensory properties of MCFAs have been studied exclusively in ethanol and water solutions (Ferreira et al. 2000, Gomez-Miguez et al. 2007). The results of the studies point to the fact that evaluation of the sensory expression of saturated fatty acids shows an even lower number of points with an increasing number of hours (weeks). Over time, sludge particles, including dead yeast with MCFAs bound, are deposited on the bottom of the vessel, and thus the typical sensory expression of these acids is fades away. This fact is very positive for wine-making practice, due to the low dosages added which should not exceed 10 mg/l (Babikova et al. 2012, Baron et al. 2017). An important limit in this experiment appears to be the 20 mg/l MCFA dose. At doses of 5, 10 and 20 mg/l, evaluators were unable to recognise a difference between these doses, while at doses of 30, 40 and 60 mg/l there was a significant increase in typical expression. In Babikova's work (Babikova et al. 2012), the negative effect of saturated MFCAs is perceived by all evaluators only at a dose of 30 mg/l or higher. Differences between the values of this study and the work of the Babikova can be caused, for example, by different varieties, the temperature of the wine and the glasses, as well the subjective response of the evaluator playing an important role.

CONCLUSION

The aim of the study is to observe the MCFA's behaviour after being added to wine and their influence on the sensory properties of the wine. The results of this experiment (MCFA residues) show that approximately 40% of MCFA content is bound at a dose of 5 mg/l, but more than 85% of MCFA is bound at a dose of 60 mg/l, therefore increasing the dose of MCFA also increases the rate of MCFA absorption by yeasts. This applies to both octanoic acid and decanoic acid. Sensory analysis was an important part of the experiment. This shows an MCFA dose of 20 mg/l as the limit above which we are able to recognise the differences in MCFA doses. At a dose of 20 mg/l the expression of saturated fatty acids is minimal. There is also the influence of sludge particles. During maturation, sludge particles with MCFA–bound yeast cells are deposited, and thus their sensory effect is reduced. Due to the low doses of MCFA used, this is positive for wine makers.

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