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Saturated higher fatty acids as a means of inhibiting alcoholic fermentation and sulphur dioxide reduction in wine

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The objective of this paper is the inhibition of alcoholic fermentation and the possibility of sulphur dioxide reduction in wine technology and wine storage. For this purpose different mixtures of the higher fatty acids (HFA) C_8, C_{10} and C_{12} were tested. Experiments confirmed that the addition of HFA into the fermenting media causes a rapid inhibition of the metabolic activity of yeasts and in consequence their dying off and a complete stop of alcoholic fermentation. The percentage of dead cells after 24 hours in wine treated with HFA (48.5 %) was almost double compared to the untreated variant (25.4 %). Treatment with a combination of HFA and 60 mg/l of sulphur dioxide gave even better results (depending on amount of HFA: 32.9 % to 77.0 %). The best effects of inhibiting yeasts and lactic acid bacteria in wine were achieved with a mixture of HFA C_8, C_{10} and C_{12} at the ratio of 2:7:1. This mixture was dissolved in 70 %vol. ethanol with a concentration of 10 g per 100 ml. Consequently the addition of HFA to must or wine can significantly reduce the dosage of other preservatives such as sulphur dioxide and sorbic acid. This method can effectively reduce the costs for the production of wines with residual sugar.
Keywords: yeast inhibition, octanoic acid, decanoic acid, dodecanoic acid, Saccharomyces cerevisiae, preservatives, sulphur dioxide

Gesättigte höhere Fettsäuren als ein Mittel zur Unterbrechung der alkoholischen Gärung und zur Reduzierung der Schwefeldioxidgehalte bei Wein. *Das Ziel dieser Arbeit ist die Unterbrechung der alkoholischen Gärung und die Möglichkeit der Reduzierung von Schwefeldioxid in der Weintechnologie und bei der Lagerung von Wein. Zu diesem Zweck wurden verschiedene Mischungen der höheren Fettsäuren (HFA) C_8, C_{10} und C_{12}getestet. Die Experimente bestätigten, dass die Zugabe von HFA in die gärenden Medien eine rasche Hemmung der metabolischen Aktivität der Hefen, und in der Folge ihr Absterben und einen vollständigen Stopp der alkoholischen Gärung verursacht. Der prozentuale Anteil toter Hefezellen nach 24 Stunden in Wein mit HFA (48,5 %) betrug fast das Doppelte der unbehandelten Variante (25,4 %). Die Behandlung mit einer Kombination von HFA und 60 mg/l Schwefeldioxid zeigte sogar noch bessere Resultate (abhängig von der HFA-Konzentration: 32,9 % bis 77,0 %). Die besten Effekte hinsichtlich einer Hemmung der Hefe und Milchsäurebakterien in Wein wurden mit einer Mischung aus den HFA C_8, C_{10} und C_{12} im Verhältnis von 2:7:1 erreicht. Diese Mischung wurde in einer Konzentration von 10 g pro 100 ml in 70 %vol. Ethanol gelöst. Der Zusatz von HFA zu Most oder Wein kann also die Dosierung anderer Konservierungsmittel, wie Schwefeldioxid oder Sorbinsäure, deutlich reduzieren. Diese Methode kann die Kosten für die Erzeugung von restsüßen Weinen effektiv reduzieren.*
Schlagwörter: Hefehemmung, Octansäure, Decansäure, Dodecansäure, Saccharomyces cerevisiae, Konservierungsstoffe, Schwefeldioxid

Les acides gras supérieurs saturés en tant que moyen d'interruption de la fermentation alcoolique et de réduction des teneurs des vins en dioxyde de soufre. *Le but du présent travail est l'interruption de la fermentation alcoolique et la possiblité de réduire la teneur en dioxyde de soufre dans la technologie des vins et au cours du stockage du vin. À cette fin, on a testé différents mélanges d'acides gras supérieurs (HFA) C_8, C_{10} et C_{12}. Les essais ont con-*

firmé que l'ajout de HFA dans les milieux en cours de fermentation entraîne l'inhibition rapide de l'activité métabolique des levures et, par la suite, le dépérissement de ces dernières et l'arrêt total de la fermentation alcoolique. Le pourcentage de cellules de levure mortes après 24 heures dans le vin contenant des HFA (48,5 %) était presque deux fois plus élevé que dans la variante non traitée (25,4 %). Le traitement aux HFA associés à 60 mg/l de dioxyde de soufre a même donné de meilleurs résultats encore (en fonction de la concentration d'HFA : de 32,9 % à 77,0 %). Quant à l'inhibition de la levure et des bactéries lactiques dans le vin, les meilleurs effets ont été obtenus à l'aide d'un mélange des HFA C_8, C_{10} et C_{12} , le rapport étant de 2:7:1. Ce mélange d'une concentration de 10 g par 100 ml a été dissous dans dans de l'éthanol de 70 %vol. L'ajout des HFA dans le moût ou le vin peut donc réduire considérablement le dosage d'autres agents conservateurs tels que le dioxyde de soufre ou l'acide sorbique. Cette méthode permet de réduire efficacement les frais de production de vins contenant du sucre résiduel.

Mots clés : inhibition de la levure, acide caprylique, acide caprique, acide laurique, Saccharomyces cerevisiae, agents conservateurs, dioxyde de soufre

The issue of a timely stopping of the alcoholic fermentation in order to produce a wine with residual sugar is very complicated. Current methods used in practice, such as cooling and filtration, lead to increasing costs and are undoubtedly very laborious and, moreover, especially for home winemakers unavailable. Separate application of sulphur dioxide is not always reliable, and at high concentrations it leads to a reduction of quality and healthiness of the wines. Moreover sulphur dioxide may cause pseudoallergic reactions therefore its dosage should be reduced in future wine technology.

An alternative product which could be used for the reduction of microbiological activity stopping fermentation is dimethyl dicarbonate (DMDC). Already more than twenty years ago Ough et al. (1988) demonstrated that 100 mg/l DMDC sterilized wine completely at pH-values below 3.8 in the absence of SO_2, even if the initial yeast population was higher than 10^7 cells per milliliter. Dimethyl dicarbonate breaks down to form carbon dioxide and methyl carbamate, which is considered to have practically no toxic effects in combination with ethanol. In the European Union DMDC is currently authorized for use in unfermented beverages at dosages below 250 mg/l. The application of DMDC is also permitted for wine, but it is restricted to the bottling process and to wines with a higher sugar content than 5 g/l. Recent results have shown that the combined application of DMDC and reduced amounts of sulphur dioxide is effective to stop fermentation and leads to a reduction of sulphur dioxide in the finished wine (Costa et al., 2008; Eder et al., 2011). With regard to these results the usage of DMDC was widened by the OIV General Assembly in Porto 2011 for stopping alcoholic fermentations.

Higher fatty acids (HFA) like octanoic acid (caprylic acid, C_8), decanoic acid (capric acid, C_{10}) and dodecanoic acid (lauric acid, C_{12}) belong to a group of higher monocarboxylic saturated fatty acids that occur naturally in walnuts or breast milk (Moltó-Puigmartí et al., 2011). These acids are industrially used for the manufacturing of dyes or perfumes. In enology their inhibitory effect on alcoholic and malolactic fermentation was studied many years ago (Laure et al., 1986; De Felice et al., 1993). Some of the higher fatty acids, like C_{16} and C_{18} acid (palmitic and stearic) are fermentation activators. On the contrary, other HFA with shorter chain, in particular acids with 6, 8 and 10 carbon atoms (C_6, C_8 and C_{10}) have fungicidal properties (Viegas und Sá-Correla, 1995; Viegas und Sá-Correia, 1991). They are formed by yeasts themselves during alcoholic fermentation and may contribute to difficulties in completing the course of fermentation (Sà-Correia, 1986). The increased concentration of short-chain HFAs is very often accompanied by problems during alcoholic fermentation. These possible inhibitive properties combined with their complete harmlessness led to the idea that they could serve as a substitution or complementation of sulphur dioxide in wine stabilization.

It seems that there exist various possibilities for the usage of HFA, but the total amount added to the wine should not exceed 10 mg/l. Since HFA have fungicidal effects they complement very well the effects of sulphur dioxide. For instance it was shown that 9 mg/l of HFA and 150 mg of SO_2 are as effective as 250 mg of SO_2 per liter alone for stopping the alcoholic fermentation of sweet wines (Ribéreau-Gayon et al., 2006). HFA should be added to the wine 24 hours before the addition of sulphur dioxide to achieve an effective reduction of living yeast cells (Laure et al., 1986).

The big advantage of this approach is that HFA added to wine are absorbed and assimilated by yeasts cells and little is esterified. In fact, studies comparing the concentration of HFA and their esters in wine treated

and untreated with HFA showed that the values remained within the normal range of 2.6 to 12.4 mg/l (fatty acids) and of 0.2 to 0.81 mg/l (ethyl esters), without any increase in the concentration of volatile compounds (Viegas et al., 1989). The majority of HFA are eliminated by fixation to the cell walls of the dead yeast cells. After such treatments, sweet wines can be preserved with only 40 mg/l of free SO_2. That means that HFA is a more effective agent to stop alcoholic fermentation than sorbic acid, which does not decrease the concentration of SO_2 used (Ribéreau-Gayon et al., 2006).
The inhibitory effect of HFA on yeasts is inversely correlated with the pH-value in a range from 3.0 to 5.4 (Viegas und Sá-Correia, 1997). This observation leads to the hypothesis that the inhibition of microbes is caused by the undissociated form of the HFAs (Alexandre et al., 1996). Moreover, it was found that dosages sufficient to inhibit microbial activity vary with the tested type of yeast, e.g. *Kluyveromyces marxianus* (EC Hansen) are less sensitive than Saccharomyces cerevisiae (EC Hansen) (Sá-Correia, 1986). The mechanism of this inhibition is not yet fully understood. The most probable theory is the passive diffusion of HFA through the plasma membrane of yeasts. As undissociated molecules the HFAs are dissolved in the phospholipid membrane and deactivate other lipid-lipid and lipid-protein interactions, resulting in a fatal modification of a specific arrangement of the membrane. This modification represents an increase in the permeability and the subsequent change in the production of enzymes and transport systems. Such affected yeasts are unable to continue to convert glucose into ethanol and finally will die.
Although the longer decanoic acid (C_{10}) is more toxic than caprylic acid (C_8) its effective toxicity is actually lower than predicted because it is badly soluble. The explanation for this phenomenon is that lipid-soluble substances with a size larger than a certain critical chain length may be considered as less toxic to the plasma membrane than smaller molecules. Moreover, at low concentrations fatty acids with longer chains have the tendency to clot formation (Lafon-Lafourcade et al., 1984).
Lafon-Lafourcade et al. (1984) showed that a combination of different HFAs (C_6, C_8 and C_{10}) added at a dose of 3 mg/l each achieved a rapid suppression of yeasts. The combination is actually more toxic than the same amount of any acid separately. In four days the number of yeast cells decreased from 8 x 10^6 to 4 x 10^4 cells per ml. Coincidentally the degradation of sugars was stopped totally. Only after a few weeks of leaving the wine in contact with the sediment, the yeast population increased again and restarted fermentation. This delay in yeast activity is probably due to their adaptation and especially esterification of HFAs. Esters are known to have much lower inhibitory effects than the free acids from which they are derived.
HFA are currently not used to inhibit the activity of yeasts during alcoholic fermentation and of lactic acid bacteria during fermentation. Publications about HFA mainly demonstrate their properties and most experiments were performed in synthetic media, not in musts or wines. Reports comparing the effectiveness of HFAs and the development of wine treated this way in the context of sulphur dioxide and the possibility of restarting fermentation (secondary fermentation) are widely missing

Materials and Methods

Fermenting must

The effectiveness of HFA to stop alcoholic fermentation was tested with a fermenting must of the variety 'Riesling Italico' with a pH-value of 3.12 and a sugar content of 212 g/l.
With the goal of determining the effects of different HFAs under variable conditions a series of experiments was performed. First of all the inhibitory effect of various inhibitory agents at various concentrations were tested in an experiment comprising twelve variants (Table 1). At a concentration of approximately 80 g/l of residual sugar the fermenting must was divided into twelve variants and the various inhibitory substances were applied. Variant 1 was without addition of an inhibitor and served as a control, in variants 2 to 5 dimethyl dicarbonate (DMDC) was used which is known for its sterilizing effect. In variants 6 to 12 mixtures of HFA consisting of C_8, C_{10} and C_{12} were applied. Each variant was performed in a volume of five liters and at a temperature of 14 °C.
The inhibitory effects of HFAs on yeasts were measured by adding different amounts (0, 5, 10 and 40 mg/l) of a mixture of HFA (C_8:C_{10}:C_{12} = 2:7:1) into the fermenting medium at 50 g/l residual sugar. This experiment was carried out without and with addition of 60 mg/l SO_2 24 hours after the addition of the HFA mixture. In an additional experiment mixtures of HFA (C_8:C_{10}:C12 = 2:7:1) with different amounts of SO_2

were added to the same fermenting grape must. Each variant was performed in a volume of 10 liters and with one duplicate.
Further experiments were carried out to identify residues of HFA in the must or wine. One variant served as a control (only 100 mg/l SO2), a second variant was treated with 10 mg/l of a mixture of HFA (C_8, $C_{10,}$ C_{12} = 2:7:1) and a last variant was treated with 10 mg/l nonanoic acid (C_9). Each variant was performed in a volume of 350 liters. Subsequently, the finished wines after the first racking from the lees were measured by means of GC-MS (Gas Chromatography/Mass Spectrometry).
To determine the development of wine treated and untreated with HFA, an experiment with already finished wine ('Malverina' variety; pH = 3.22; 18 g/l residual sugar; sterile unfiltered) was carried out with dosages of 100 and 150 mg/l of SO_2 and individual fatty acids (all 10 mg/l), 100 mg/l of SO_2 (Table 3.). The experiment was carried out at a 10 l-scale with one repetition. No further protection against oxidation was performed and the storage temperature was 25 °C. The number of microorganisms was determined by direct counting in a Bürker counting chamber. The samples were diluted to give a number of microorganisms ranging from 10 to 40 cells per square. On the cleaned glass of the counting chamber a fermenting must sample treated with methylene blue was pipetted for easy differentiation of viable and dead cells. Under the microscope (Olympus CX 31) at a magnification of 600, each field of the Bürker counting chamber was monitored. 72 fields were evaluated at six columns to twelve squares. Each square has an area of 1/25 mm^2. The counting was carried out both at the upper part and the lower part of the chamber. The difference between the counted numbers of cells in the upper and lower part of the chamber should be less than 10 %.

Results and Discussion

The results show (Table 1) that the fastest inhibitory effect was clearly achieved with the addition of DMDC. Its addition stopped the conversion of sugar almost immediately. In the case of HFA addition the inhibition of alcoholic fermentation was more slowly, but also achieved. The highest inhibitory effect was found with DMDC at concentrations of 100 to 200 mg/l, which is consistent with the report of Ribéreau-Gayon et al. (2006). Also a strong inhibitory effect of HFA was shown in variants 7 and 8. Each value represents the mean of two measurements.

Table 1: Monitoring of residual sugar after inhibition of alcoholic fermentation

Var.	Used compound (mg/l)	Residual sugar content (g/l)		
		Measuring date 2010		
		4.5.	18.5.	Diff.
1	Control	49	21	28
2	50 DMDC	68	40	28
3	100 DMDC	61	60	1
4	150 DMDC	77	76	1
5	200 DMDC	77	76	1
6	10 C8	48	34	14
7	10 C10	55	49	6
8	5 C8 + 5 C10	55	45	10
9	5 C8	54	39	15
10	5 C10	50	34	16
11	5 C12	49	36	13
12	10 C12	59	43	16

To confirm the hypothesis that HFA has an inhibitory effect following microbiological tests were conducted. Different amounts (0, 5, 10 and 40 mg/l) of a mixture of HFA (C_8:C_{10}:C_{12} = 2:7:1) were added into the fermenting medium at 50 g/l residual sugar. (Fig. 1A). The ratio of HFA was established on the basis of previous experiences in order to have a maximum inhibitory effect on yeast cells while preventing malolactic fermentation. The different solubility of the HFAs, which is dependent on chain length was taken into account. Figure 1B shows the effects of the same doses of HFA, but in this case with the application of 60 mg/l SO_2 24 hours after the addition of the HFA mixture.
In Figure 1A the number of dead yeast cells after addition of the HFA mixture without SO_2 is shown. It is evident that the application of HFA without SO_2 leads to a doubling of dead cells in the medium. Also a gradual stopping of alcoholic fermentation could be observed. This trend was maintained even after seven days, with approximately 90 % of dead yeasts in the medium after the application of the highest dose of HFA.
Figure 1B confirms the synergic effect of HFA and SO_2. From these results it can be seen that already one day after treatment the amount of dead yeast cells is

very high (almost 80 %). After three days the number of dead yeast cells was comparable with the treatment with HFA solely (without SO_2) after seven days. However, it is necessary to take also into account the fact that some yeasts die naturally at the final phase of alcoholic fermentation.

When a mixture of HFA (C_8:C_{10}:C_{12} = 2:7:1) together with different amounts of SO2 was added to the fermenting grape must, it was found that in all variants with the combined application of HFA and SO_2 lower levels of free SO_2 (average about 3.8 mg/l) and lower

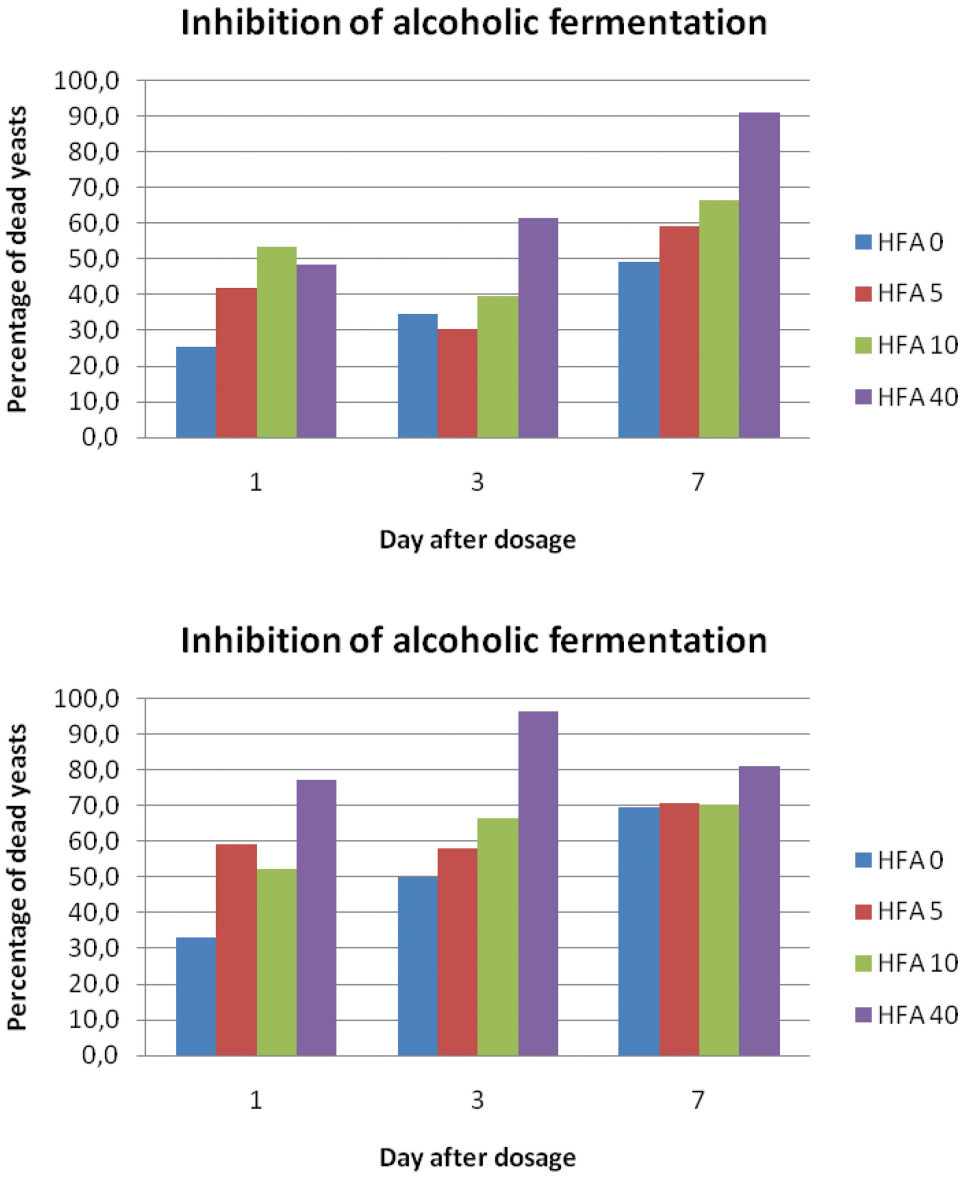


Fig. 1: Monitoring of dead yeast population after HFA dosage – up (**A**), after HFA + 60 mg/l SO_2 dosage – down (**B**)

levels of total SO_2 were measured (Table 2). The explanation for this observation is probably the higher consumption of SO_2, because it can penetrate better and more effectively into the bodies of yeast cells which are already inhibited by HFA. This causes a higher uptake of SO_2 which results in a higher percentage of dead yeast population.

wine technology is very disadvantageous because of its non-physiological nature.

Since a large portion of HFAs are fixed to the yeast cells after application and consequently eliminated during clarification the organoleptic properties of wines treated this way is negatively affected (Ribéreau-Gayon et al., 2006).

The experiment with the already finished wine ('Malverina') showed, that the variants treated with HFA (all 10 mg/l, 100 mg/l of SO_2) had a relatively equal development of the sulphur dioxide content down to concentrations of about 25 to 30 mg/l compared to the variants treated with 100 and 150 mg/l of SO_2. This means, that at such a late addition of HFA the yeasts are likely involved more and more. They quickly begin to consume the SO_2, because they are not longer inhibited by it. In the case of the HFA treated variants, however, the concentration of viable yeasts in the medium stays much lower and thus the wine is more resistant to re-fermentation. Even in the sample trea-

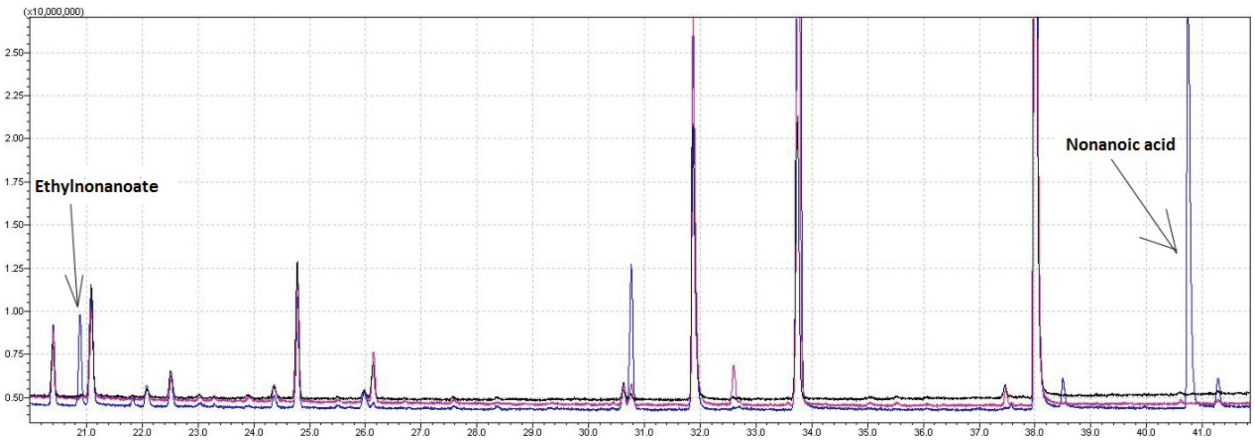
Table 2: Monitoring of SO_2 concentration in treated and untreated wines

Variant	SO_2 (mg/l)	SO_2 + HFA		SO_2 without HFA		Free SO_2 Difference	Total SO_2 Difference
		Free SO_2	Total SO_2	Free SO_2	Total SO_2		
1	0	6,3	29,1	10,1	31,6	3,8	2,5
2	20	12,7	45,6	16,6	48,3	3,9	2,7
3	40	25,3	64,5	29,1	72,1	3,8	7,6
4	60	40,5	88,6	44,3	93,6	3,8	5,0
5	80	53,1	98,7	56,9	107,6	3,8	8,9

Note: The values of SO_2 are measured by iodometric determination including ascorbic acid and reductions

Figure 2 shows the result of the gaschromatographical analysis of residues of HFA in wines after treatment. The pink chromatogram represents the control sample (only 100 mg/l SO_2), the black lines show the results after addition of a mixture of HFA and the blue chromatogram the results for the addition of nonanoic acid (C_9 fatty acid). This comparison revealed non-physiological peaks of ethylnonanoate (C_9 fatty acid ethyl ester) and C_9 fatty acid. When using a mixture of C_8, C_{10} and C_{12} (2:7:1), there was no qualitative effect compared with the control. After few months only a minimal increase of HFA ethylesters was detected (Fig. 2). C_9 fatty acid is indeed the last liquid at room temperature and its toxicity is very high, but its use in

Fig. 2: Qualitative comparison of wines untreated and treated with HFA



ted with a dose of 150 mg/l SO_2 under oxidative storage conditions the secondary fermentation began earlier than in the variants treated with a combination of 100 mg/l SO_2 and the individual HFA. In this way the HFA can be used for preserving wines with residual sugar and can lead to a reduced usage of SO_2.

lic fermentation. Dosage into the finished wine works as a precaution against lactic acid bacteria and secondary fermentation (re-fermenting). Addition of HFA can significantly reduce the dosage of other preservatives such as sulphur dioxide and sorbic acid. This method can effectively reduce the costs of the techno-

Table 3: Evolution of free sulphur dioxide in wines treated with HFA and SO_2

Date	Initial doses of HFA and sulphur dioxide (mg/l)				
	100 SO_2	C_8 + 100 SO_2	C_{10} + 100 SO_2	C_{12} + 100 SO_2	150 SO_2
4.8.	54,2	50,6	50,6	60,2	91,5
6.8.	49,4	48,2	48,2	55,4	85,5
9.8.	45,8	42,2	43,4	53,0	80,7
15.8.	33,7	30,1	31,3	37,3	67,5
17.8.	32,5	32,5	33,7	39,7	63,8
23.8.	22,9	25,3	26,5	31,3	55,4
30.8.	10,8	18,1	19,3	21,7	43,4
4.9.	3,2	10,8	10,8	13,2	22,3
9.9.	-	6,0	6,0	10,8	12,0
15.9.	-	1,7	1,8	2,5	-

Note: The values of SO_2 are measured by iodometric determination including ascorbic acid and reductions

CONCLUSIONS

A mixture of HFA (C_8, C_{10} and C_{12} = 2:7:1) offers optimized properties to inhibit yeasts and lactic acid bacteria. If this mixture is dissolved in 70 %vol. ethanol at a concentration of 10 g per 100 ml it has the potential to prevent secondary fermentation in wine. Such a mixture is prepared in a liquid state and it is unlikely to create a solid phase at low temperatures, which makes it very easy to dose in practice. The advantage of the proposed mix is the high fungicidal activity of C_8, C_{10} and C_{12} fatty acids and the maximum toxicity against lactic acid bacteria. The secondary purpose of caprylic acid (C_8) is to increase the solubility of the badly soluble lauric acid (C_{12}). Using HFA with chain lengths above twelve carbons is irrelevant, since they have a significant decrease of solubility in mixtures similar to wine or must and would create a useless film on the surface.

Recommended dosage into the fermenting media is 1:10000 (1 ml to 10 liters of medium) and for finished wine it is 1:20000. It was observed that these dosage rates have almost no negative influence on the organoleptic characteristics of the resulting wines. Dosage of the HFA mixture into the fermenting media causes a rapid inhibition of the metabolic activity of the yeasts, their dying off and a complete stopping of the alcohology involved in the production of wines with residual sugar. Especially effective is the addition of HFA in combination with reduced doses of sulphur dioxide under small-scale winemaking conditions where it is not possible to use expensive routine operations as applied in larger wineries (refrigeration, sterile filtration). Finally, in accordance to today's demand for sulphur dioxide reduction in wines, especially for organically produced wines, this mixture could be a very effective aid.

References

Alexandre, H., Mathieu, B. and Charpentier, C. 1996: Alteration in membrane fluidity and lipid composition, and modulation of H + -ATPase activity in Saccharomyces cerevisiae caused by decanoic acid. Microbiology 142: 469-475

Costa, A., Barata, A., Malfeito-Ferreira, M., Loureiro, V. 2008: Evaluation of the inhibitory effect of dimethyl dicarbonate (DMDC) against wine microorganisms. Food Microbiology 25: 422-427

De Felice, M. 1993: Free fatty acids inhibiting malolactic fermentation in wine. Note 1. Ind. Bevande 22: 126-130

Eder, R., Del Hierro, A. and Scheiblhofer, H. (2011): Reduction of sulphurdioxide content in natural sweet wines by usage of DMDC. 34th World Congress of Vine and Wine. OIV Section II - Oenology: E. Winemaking Technology. – Porto, 20. – 27. June 2011

Lafon-Lafourcade, S., Geneix, C. and Ribéreau-Gayon, P.

1984: Inhibition of alcoholic fermentation of grape must by fatty acids produced by yeasts and their elimination by yeast ghosts. Appl. Environ. Microbiol. 47(6): 1246-1249

Laure, F., Murakami, Y., Boidron, J.N. and Fohr, L. 1986: First observations on the use of octanoic and decanoic acids as adjuvants to sulphur dioxide for the stabilization of sweet wines: organoleptic effects. Conn. Vigne Vin 20(2): 87-95

Moltó-Puigmartí C., Castellote, A.I., Carbonell-Estrany, X., López-Sabater, M.C. 2011: Differences in fat content and fatty acid proportions among colostrum, transitional, and mature milk from women delivering very preterm, preterm, and term infants. Clinical Nutrition 30(1): 116-123

Ough, C.S., Kunkee, R.E., Vilas, M.R., Bordeu, E., Huang, M. C. 1988: The interaction of sulphur dioxide, pH and dimethyl dicarbonate on the growth of Saccharomyces cerevisiae Montrachet and Leuconostoc oenos MCW. Am. J. Enol. Vitic. 39(4): 279–282

Ribéreau-Gayon, P., Dubourdieu, D., Donéche, B. and Lonvaud, A. (2006): The microbiology of wine and vinifications. 2nd ed. – Chichester: Wiley, 2006
(Handbook of enology ; Vol. 1)

Sá-Correia, I. 1986: Synergistic effects of ethanol, octanoic and decanoic acids on the kinetics and the activation parameters of thermal death in Saccharomyces bayanus. Biotechnol. Bioeng. 28: 761-763

Sá-Correia, I. and Van Uden, N. 1983: Temperature profiles of ethanol tolerance: effects of ethanol on the minimum and the maximum temperatures for growth of the yeasts Saccharomyces cerevisiae and Kluyveromyces fragilis. Biotechnol. Bioeng. 25: 1665-1667

Viegas, C.A. and Sá-Correia, I. 1991: Activation of plasma membrane ATPase of Saccharomyces cerevisiae by octanoic acid. J. Gen. Microbiol. 137: 645-651

Viegas, C.A. and Sá-Correia, I. 1995: Toxicity of octanoic acid in Saccharomyces cerevisiae at temperatures between 8.5 and 30°C. Enzyme Microb. Technol. 17: 826-831

Viegas, C.A. and Sá-Correia, I. 1997: Effects of low temperatures (9-33°C) and pH (3.3-5.7) in the loss of Saccharomyces cerevbsiae viability by combining lethal concentrations of ethanol with octanoic and decanoic acids Int. J. Food Microbiol. 34: 267-277

Viegas, M.F. Rosa, I. Sá-Correia and Novais, J.M. 1989: Inhibition of yeast growth by octanoic and decanoic acids produced during ethanolic fermentation. Appl. Environ. Microbiol. 55: 21-28

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