TECHNOLOGIES TO REDUCE MICROBIAL SPOILAGE, OXYDATION, AND USE OF SULPHITES

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This note considers some physical technologies, which can be useful in reducing the risk of microbial contamination and wine oxidation as well as the use of SO_2. Flash-pasteurisation (FP), Cross-Flow microfiltration (CF-MF) and Electro-dialysis bipolar membrane) were evaluated to find out to what extent these technologies can be implemented in organic wine-making without affecting wine quality and production costs.
Electro-dialysis has been tested for acidification of red wine and flash-pasteurisation and cross-flow microfiltration for microbiological stabilisation against yeasts or bacteria.

ELECTRO-DIALYSIS WITH BIPOLAR MEMBRANES / ACIDIFICATION

Grape acidity development as recorded during the last years shows a regular pH progression with very high levels in all the European countries. Very high pH has led to an increase in the amounts of SO_2 used.
INRA (in relation with EURODIA) have developed the use of electro-dialysis bipolar membranes. This technique allows pH regulation (acidification). This treatment can be automated and produces a required pH final value. Thus, controlled acidification allows the production of more favourable conditions for sulphur dioxide use (active SO_2).

Principles of the Electro-dialysis bipolar membrane:

a) Bipolar membrane electro-dialysis efficiently converts aqueous salt solutions into acids and bases without chemical addition. It is an electro-dialysis process as ion exchange membranes are used to separate ionic species in solution with the force of an electrical field, but differs by the single water splitting capability of the bipolar membrane. The process also offers the capability to directly adjust the acidity of process streams without adding chemicals.
b) The wine can be acidified (Fig. 1) with the association of bipolar membrane and cationic exchange membrane. Hydrogen ions coming from the splitting of water which replace the potassium ions going out through the cationic membrane.

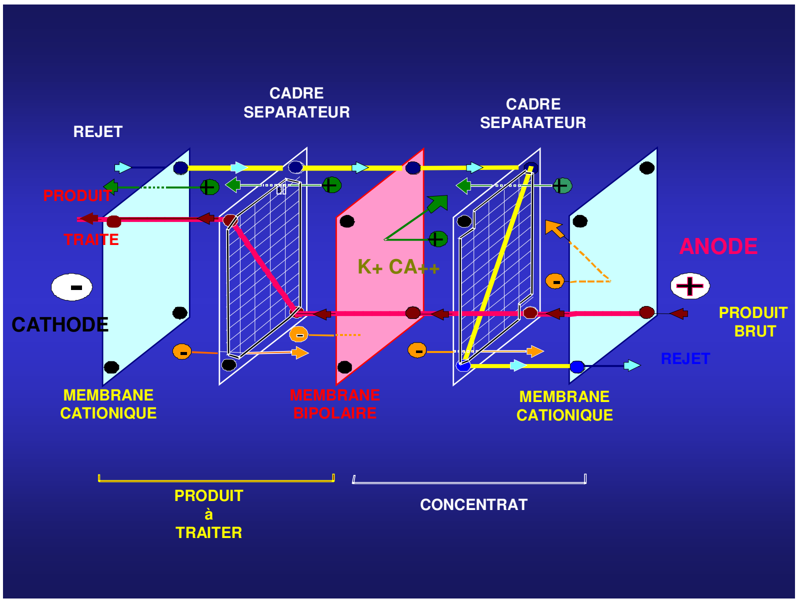


Fig. 1: Principe of electro-dialysis bipolar membrane (source: INRA)

Experimental procedure:

The acidification by this bipolar process was performed on a red wine (Syrah) with a very high pH (about 4.15). Reference acidifications were performed with two levels of tartaric acid (1,5 and 3 g/L) added for comparison. After addition of tartaric acid the wines were cooled in a cold chamber for 15 days (0°C) and were racked to eliminate the tartaric precipitate.
The wine was treated by the bipolar process with a large pH range (from 3.25 to 4.15) and with the addition of SO_2 at bottling with two levels (no addition, and 1 g/hl).
The activity of SO_2 / acidity was tested by growth of contaminant yeast (inoculation by *Brettanomyces*).

Results :

The bipolar process can accurately produce the required pH. As the theory indicates the variation of pH is linked to the substitution of K+ by H+. Tartaric acid concentrations are not different for all modalities. The acidity increased with the decrease of pH. After bottling, the differences between addition or no addition of SO_2 are very small (about 2 mg/L with addition of 1 g/hl). SO_2 added is therefore quickly bound in these wines.
The acidification with tartaric acid allowed small variations of pH; -0.15 for 1.5 g/L addition and –0.35 for 3g/L additions. In fact acidification allowed precipitation between tartaric acid and K+. The pH decrease is a consequence of the K+ concentration decrease. Tartaric acid concentrations were increased slightly.
During a 35 day period the inoculated *Brettanomyces* population was followed (Figure 2).

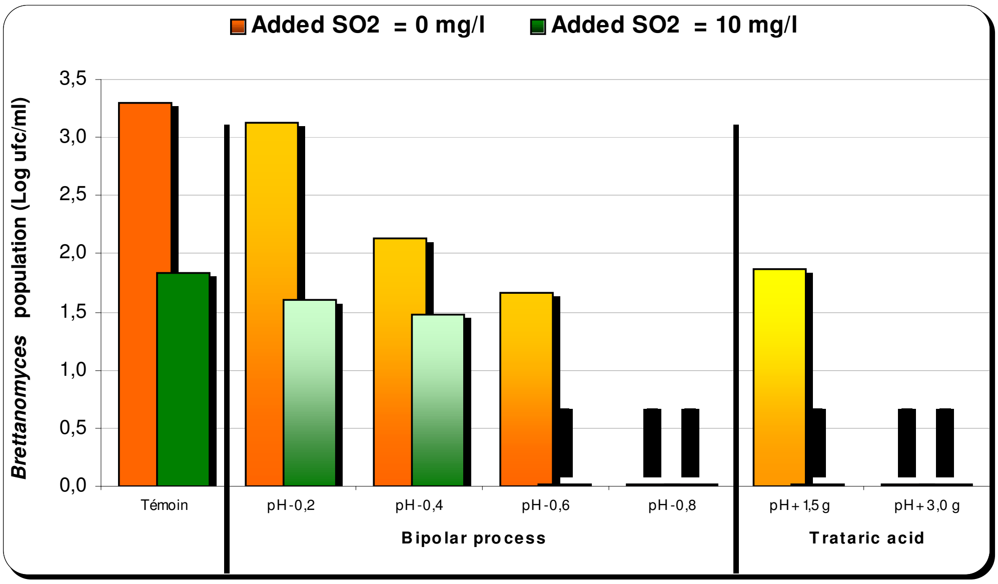


Fig. 2: *Brettanomyces* population in each treatment 3 days after contamination (average of two repetitions)

The decrease of *Brettanomyces* population is linked to the decrease of pH.
Acidification with tartaric acid is more efficient in the inhibition of *Brettanomyces* growth than bipolar process with the same pH variation with or without SO_2.
A very low SO_2 addition was much more efficient as the level of acidification was high.

The active SO_2 was directly linked to pH levels but this effect was identical in conventional or organic wines.

The present technique is not yet permitted in general wine regulations and can not be considered for at least 2 or 3 years.

FLASH-PASTEURISATION (FP), CROSS-FLOW MICROFILTRATION (CFM) FOR ENHANCEMENT OF WINES WITH RESIDUAL SUGAR

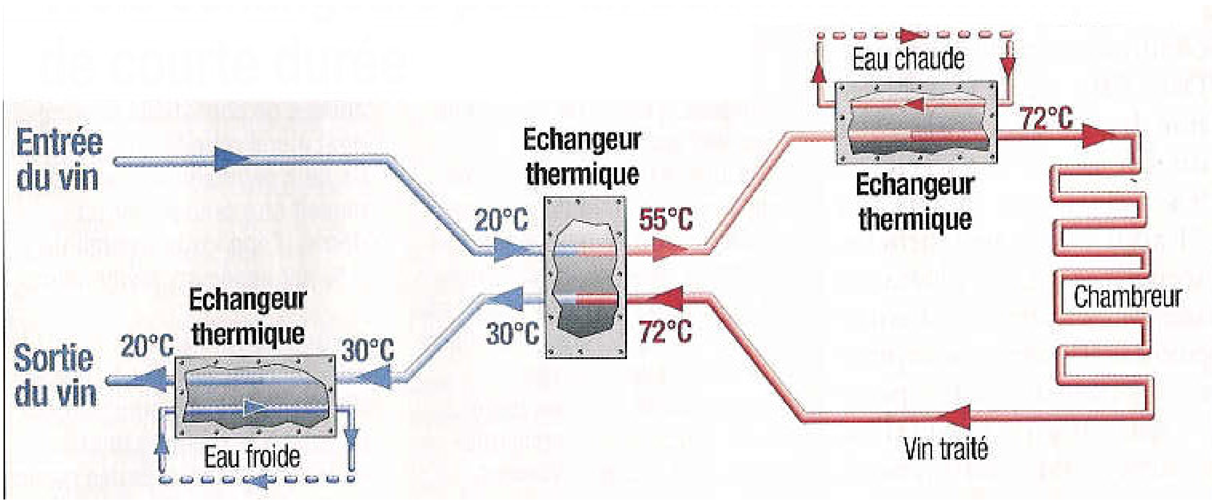
These technologies were tested for stopping alcoholic fermentation during the development of sweet wines. The comparison was carried out with the addition of SO_2 as "SO_2 mutage" and DMDC - Dimethyldicarbonate, recently permitted by EU, but not accepted for organic wine-making. The wines were developed to make white or rosé wines and the fermentations were stopped with a low alcoholic degree to reach a difficult situation of stability.

Experimental procedure :

- Grapes of mourvèdre (domaine INRA of Gruissan – 11430) were crushed and pressed to obtain a juice (14 % Vol of potential alcohol. The fermentation was stopped when the wine reached about 12% Vol). All treatments received 8 g/hl of SO_2, except for SO_2 alone treatment (5g/hl for the "mutage" + 8 g/hl SO_2 like the other treatments).

- An experimental design (in Erlenmeyer of 200 ml) was followed for each process (except DMDC) with the contamination by yeasts (S. Cerevisea K1) (3 levels: 0, 10^2, 10^4 cells by millilitre) and 4 levels of SO_2 (0, 4, 8,12 g/hl) and 2 repetitions of each treatment (trials carried out in 200 ml Erlenmeyer's until the end of fermentation – 2 x 3 x 3 x 4 = 72 tanks of 200ml – registering of weights of the Erlenmeyer's).

Fig. 3: Technical principle of flash-pasteurisation.



Results:

The analyses of the wines were almost identical. The combination of SO_2 is a little higher for the reference "SO_2". The benefit of the SO_2 control is only 20 mg/L. There were no significant differences in colour.

There is no significant difference (test 5%) between aromatic profiles, except for the SO_2 control where panelists found bad smells. Consequently the quality of this treatment is lower than for the other wines.

The acidity of SO_2 control is lower than the others (no differences in the analysis). The body intensity of the DMDC treatment seems to be lower than for other wines (near 5%). The other characteristics are not significant.

The level of overall pleasantness is significantly higher for CFM wine in comparison with the SO_2 treatment (bad smells) and the two other wines were in between.

After 5 months in the Erlenmeyer test there are only 2 treatments with SO_2 stabilisation. Where fermentation is still preceding whatever the level of the yeast population 8 g/hl of SO_2 are necessary to stop the re-fermentation.
In the other cases, fermentations can start up again on an uncertain basis and there is no link with yeasting.

The effect of mutage with CFM and FP is very strong. It's possible to reduce SO_2 without fermentation risks with these technologies.

Fig. 4: Technical equipment for flash-pasteurisation

These technologies can produce good microbiological stabilisation but the combination of the SO_2 is the same as the control. If there is a need to obtain the same concentration of free SO_2 in these different final wines, the reduction in the amount of SO_2 to be added is very low (about 20 mg/L in these experiments).

DMDC seems to represent a good alternative for "mutage" in replacement of SO_2. But the chemical origin of this product seems to not conform in an organic way.
The sensory test has shown that CFM wine is the best wine in this trial. The different tested technologies do not change sensory profiles of wines.

FLASH-PASTEURISATION (FP), CROSS-FLOW MICROFILTRATION (CFM) FOR BACTERIA STABILISATION

These technologies were tested for stopping lactic bacteria fermentation during the making of white wines. The difference between SO_2 and Lysozyme additions was assessed.

These technologies were tested for red wines after lactic bacteria fermentation but before wine ageing and storage. Again the difference between SO_2 and Lysozyme additions was assessed.

White wine experimental procedure:

Organic white wine was selected in a cellar (organic winery) just after the end of alcoholic fermentation. Four treatments were studied (SO_2, Lysozyme, Flash-Pasteurisation, Cross-Flow Microfiltration) with 2 levels of SO_2 concentration at bottling.

An experimental laboratory procedure was followed for each process, with controlled contamination of lactic bacteria at different inoculum levels, and with different levels of free SO_2 (0, 10, 30 mg/L).

Results:

The wine analyses were very similar with the exception of acidity. The SO_2 and FP treatments resulted in a lower concentration of tartaric acid. The precipitation of both tartaric acid and potassium ions was more efficient for these treatments.

The combination of SO_2 is a little higher for the control "SO_2" but only for the "high SO_2" treatments. The net benefit of SO_2 usage is only about 10 mg/L.
In the sensorial testing, there is only one 5% significant difference. The treatments: FD low SO2 and high SO2, CFM low SO2 and high SO_2. High levels are less 'vegetal' than the other wines. The other differences are not significant.
The Lysozyme treatments appear to exhibit a more aromatic intensity but there is no preference between the different wines.
There appears to be a difference between the wines, but there is no link with the applied treatments (similarly for bitterness). For overall quality, the CFM treatments gave the lowest scores.

In the results of laboratory tests (table 1), there were no differences between the treatments with bacterial inoculation, except for the Lysozyme samples where the inoculation was not adequate to induce lactic bacteria fermentation. With bacterial inoculation, it seems that FP and the CFM treatments were a little more unstable from a microbiological point of view. However, due to the length of the experiments, these results might have resulted from accidental contamination.

Table 1: Results of bacterial inoculation – White wines – IFV ORWINE 2007-2008

FML duration (days)	CFM			FP			SO_2			Lysozyme		
SO_2 modalities	0	10	30	0	10	30	0	10	30	0	10	30
Bacteria 0	> 90	N	N	90	N	N	N	N	N	N	N	N
Bacteria 10^2 cfu/ml	90	N	N	45	> 90	N	50	N	N	N	N	N
Bacteria 10^5 cfu/ml	40	70	N	30	60	N	40	80	N	N	N	N

Red wine experimental procedure:

Organic red wine was selected from an organic winery just after the end of malolactic fermentation. Four treatments were studied (SO_2, Lysozyme, Flash-Pasteurisation, Cross-Flow Microfiltration) with 2 levels of SO_2 concentration at bottling (0 and 2 g/hl).

Results:
Wine analyses were very similar for all treatments tested. K^+ and tartaric acid concentrations are lower for SO_2 and Lysozyme treatments. The final concentrations of SO_2 in the different treatments are lower than expected. The combination with SO_2 is higher than expected for all treatments. There are no significant differences on colour or 'vegetal' assessments between the treatments. It seems that the "vegetal" parameter is higher for certain treatments but without clear linkage with the technologies used.
It is the same for the gustative parameters, except for the tendency of global quality. The best scores are obtained with CFM modalities.

Conclusions:
The technologies used in these trials are able to stabilise the tested wines. In all cases, there is a reduction of the quantity of SO_2 needed (it is possible to completely avoid SO_2 use).
For total control of bacteria the use of Lysozyme is the only alternative to $SO_{2\ l}$ in order to avoid malolactic fermentations even after inoculation or contamination with bacteria.
If the objective is to reach a certain concentration on free SO_2 after bottling, it should be noted that all tested alternatives give wines with the same concentration of total SO_2. SO_2 combinations are near the same in all treatments. With such technological alternatives it is therefore only possible to decrease the concentration of total SO_2 about 10 to 20 mg/L.

Wines without free SO_2 often exhibit oxidised olfactory profiles. The tested alternatives (chemical or physical) cannot replace the specific action of SO_2 (protection against oxygen). A strict hygienic control and an efficient bottling process are necessary to achieve a reduction of free SO_2 concentration.
A new analytical approach using sensitive crystallisation can be carried out which will give a better assessment of the tested technologies.
This approach involves the reading of crystallizations in Petri boxes and is not easily adapted to standard scientific testing. The final interpretation is more literary than scientific! In these trial experiments, the expertise of Margaret Chapelle who has worked on such analyses for 25 years should be used.

Thus in conclusion these technologies did not make a lot of differences on the final tested wines. The only differences are in relation with Flash-Pasteurisation treatments on white wines. The pictures of crystallization of these treatments are very different from the others. The explanation concerns the "life of wine" (swift time of aroma).

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